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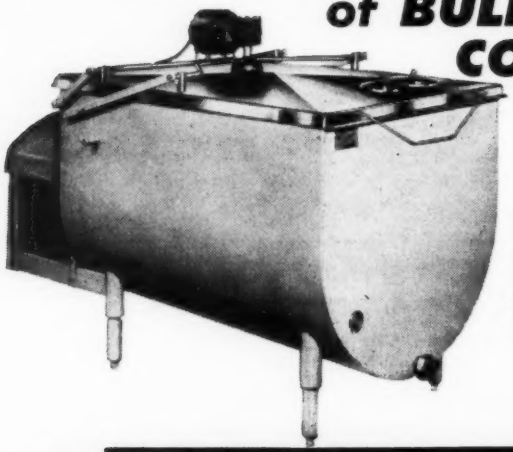

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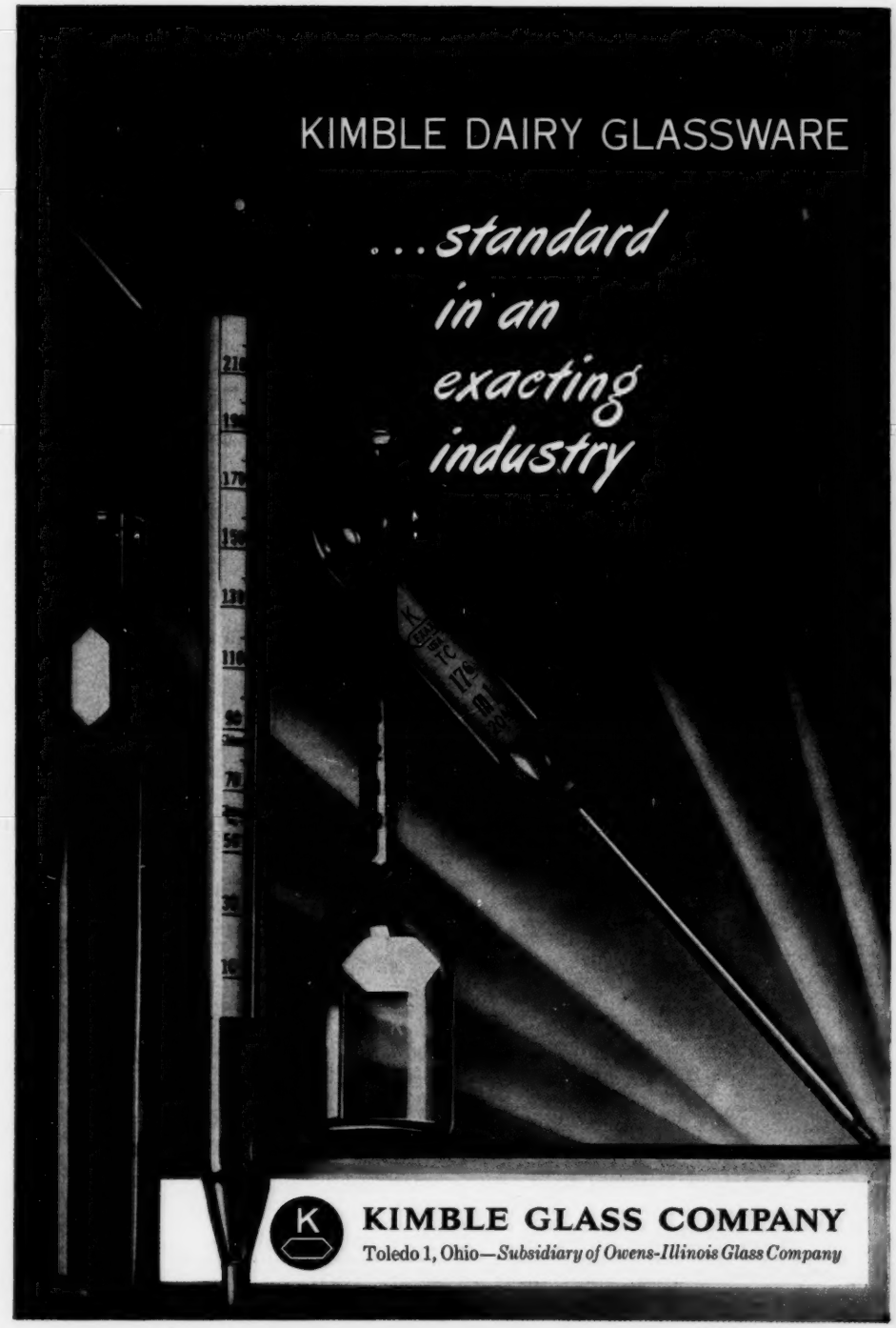

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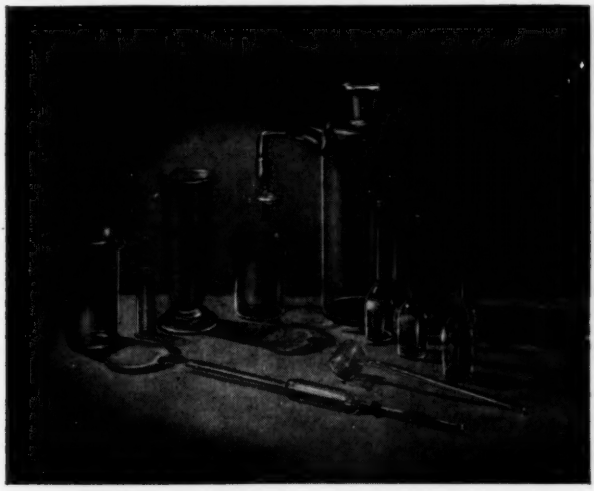

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INTERRELATIONSHIPS BETWEEN SITE OF DEPOSITION, DOSAGE, AND NUMBER OF SPERMATOZOA IN DILUTED SEMEN AND FERTILITY OF DAIRY COWS INSEMINATED ARTIFICIALLY[1]

DURWARD OLDS, D. M. SEATH, M. C. CARPENTER,[2] AND H. L. LUCAS[3]

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Three factors of fundamental importance to the technique of inseminating cattle are: where semen should be deposited, what dosage should be used, and what concentration of spermatozoa should be in the diluted semen.

Salisbury and Bratton (8) found no significant differences in the fertility of semen diluted from 1:100 to 1:400. However, when the number of spermatozoa per insemination varied from 15.3 to 2.36 million, there was a downward trend of 0.8% in fertility for each decrease of 1 million spermatozoa. They suggested that the minimum number of spermatozoa consistent with optimum fertility was 5 to 10 million per insemination. Willett (13) also reported a downward trend in fertility with each increase in dilution above 1:100. When the number of spermatozoa per insemination decreased from approximately 12 million to 6 million, there was a decrease of 0.5% in fertility for each million decrease in spermatozoa. When the diluted semen contained less than 6 million spermatozoa per milliliter there was a drop of 2.6% in fertility for each million decrease in number of spermatozoa. Branton *et al.* (1) found no significant differences in the fertility of diluted semen varying from 4 to 16 million spermatozoa per milliliter.

Regarding the site of depositing semen, Lasley and Bogart (5), Holt (3), and Raps (6) have shown that intra-uterine insemination results in higher fertility than cervical insemination by the speculum method. Recent and more extensive studies by Salisbury and VanDemark (9), Knight *et al.* (4), and Weeth and Herman (12) have shown no significant difference in fertility between intra-uterine and intracervical insemination using the recto-vaginal technique. However, Weeth and Herman (12) found a higher fertility with intra-uterine insemination on repeat services and with semen 24 to 36 hours old. Furthermore, VanDemark *et al.* (11) have shown that mid-cervical in-

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

[2] Manager, Kentucky Artificial Breeding Association.

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semination of pregnant cows does not interrupt pregnancy, whereas intra-uterine insemination may cause either abortion or fetal death and decomposition. The use of antibiotics in the semen reduced this hazard but did not completely remove it. Donald (2) has reported that about 3.4% of the cows showed estrus one or more times while pregnant.

Very little information is available regarding the proper dosage of diluted semen for inseminating cattle. As cited above, considerable work has been done on the number of sperm to be used, but it is not known whether the amount of diluting fluid used to carry the desired number of spermatozoa has a bearing on fertility or not. Bull studs, in expanding their services, are often faced with the problem of deciding whether they should increase their dilution rate, request inseminators to use smaller dosages, or add more bulls to their studs.

EXPERIMENTAL PROCEDURE

Inseminators in 16 local cooperatives of the Kentucky Artificial Breeding Association were selected to take part in the study for a period of 4 months, starting in January 1952. Inseminators were selected after considering their ability, willingness to cooperate, length of service, fertility obtained, and number of cows being bred. There were 28 locals not on experiment.

The four sites of depositing semen were: (a) half-way through the cervix, (b) body of the uterus, (c) both uterine horns, and (d) both uterine horns, body of the uterus, and the cervix. Each inseminator was assigned a site of deposition which he used throughout the 4-month period. There were four inseminators using each site.

Four dosages of semen were used as follows: 0.25 ml., 0.5 ml., 1.0 ml., and 2.0 ml. Intradermal tuberculin syringes, graduated in 0.01 ml., were used for measuring the 0.25 and 0.5 ml. doses; heifer-size plastic inseminating tubes having a 1-mm. bore were used. For the 1.0 and 2.0 ml. doses, regular 2-ml. syringes were used with either heifer- or cow-size inseminating tubes. All dosages were based on semen delivered rather than on volume of semen drawn up into the tube by the syringe. The proper amount to draw up for each dosage was determined by weighing the amount which could be delivered. Syringes then were marked to facilitate their use by the inseminators. It is believed that this procedure held errors in dosage to a reasonable minimum. Each dosage was used by four inseminators each month. The dosages were alternated so that all inseminators used each of the dosages for one month during the experimental period.

The concentration of spermatozoa in the semen used by the 16 experimental locals was controlled at the bull stud. All ejaculates collected for each shipment from a bull were mixed together, and 0.15 ml. of this pooled sample was added to 1.35 ml. of 2.9% sodium citrate dihydrate, thus making a dilution of 1:9 for use in micro-tubes of a Klett-Summerson colorimeter. The colorimeter was standardized by making serial dilutions of six different semen samples which had been counted at least four times in a hemacytometer. The curve thus obtained was used in estimating the concentration of all ejaculates

from the colorimeter reading. Based on previous work by Willett and Buckner (14) and Salisbury *et al.* (7), this method was considered sufficiently reliable for practical purposes.

Half of the locals were sent semen containing an estimated 24 million spermatozoa per milliliter and the other half were sent semen containing an estimated 16 million per milliliter. This was alternated each month so that each dosage and each site would be used an equal number of months with each concentration of semen. Numerous hemacytometer counts made during the course of the experiment indicated that the average error in concentration of spermatozoa was not more than 2 to 3 million spermatozoa per milliliter of diluted semen.

The statistical design permitted employing multiple covariance in the analysis to remove the effects of differences in normal fertility obtained by inseminators as measured by their rating for the month prior to the experiment and the over-all differences between the 4 months during the experiment. Analysis of variance then was used to test the effects of the different methods of insemination.

RESULTS

The multiple covariance analysis indicated that neither inseminator's previous fertility rating nor monthly variations in fertility had significant effects on results obtained during the experimental period. Therefore, the experimental results were analyzed by the analysis of variance without adjustment.

TABLE 1

Percentage of 60 to 90 day nonreturns from first service obtained by using two concentrations, four sites of depositing, and four dosages of diluted semen [a]

Concentration 24 million per ml.	Site of Depositing Semen				
Dosage *(ml.)*	Horns, body, and cervix	Horns	Body	Cervix	Av.
2.0	73.4	73.4	67.7	66.0	70.1
1.0	69.3	68.6	67.4	72.0	69.3
0.5	75.4	67.0	68.8	65.4	69.1
0.25	59.2	66.2	63.7	67.1	64.1
Av.	69.3	68.8	66.9	67.6	68.2
16 million per ml.					
2.0	68.7	65.7	68.2	72.7	68.8
1.0	73.4	65.6	63.9	55.4	64.6
0.5	66.0	66.1	59.1	72.1	65.8
0.25	66.1	71.0	66.7	58.7	65.6
Av.	68.6	67.1	64.5	64.7	66.2
Av. for site of depositing	69.0	67.9	65.7	66.2	67.2

Av. for dosage *(ml.)*	% 60-90 day N-R
2.0	69.5
1.0	67.5
0.5	67.3
0.25	64.7

[a] The number of cows bred with each treatment varied from 174 to 435 and averaged 298.7.

The average fertility (60 to 90 day nonreturns for first services) obtained for each concentration, site of deposition, and dosage of semen is shown in Table 1. Each percentage figure represents the average of two locals which were replicates of each other and is based on an average of 298.7 cows. The analysis of variance (Table 2) indicates that the differences found in nonreturns due to the various treatments were in each comparison too small to be statistically significant. There was a tendency, however, for the larger dosage of semen (2 ml.) to produce a higher nonreturn rate than the smaller dosages. Also, the distribution of the semen into the horns, the body, and the cervix produced the highest rate of nonreturns (69.0%). Likewise, the more concentrated semen,

TABLE 2

Analysis of variance of data resulting from the use of two concentrations of spermatozoa in diluted semen, four sites of deposition, and four dosages and their effect on cows showing nonreturns for service

Source of variance	D.F.	Sum of squares	Mean square
Total	63	2,791.40	
Concentrations	1	61.20	61.20
Sites	3	111.32	37.11
Dosages	3	172.06	57.35
Conc. × site	3	10.48	3.49
Conc. × dosage	3	89.41	29.80
Site × dosage	9	228.64	25.40
Conc. × site × dosage	9	653.33	72.59
Error	32	1,464.93	45.78

24 million per milliliter, averaged 2.0 percentage points higher in nonreturns than did the semen containing 16 million per milliliter. The analysis of variance shows that the three-way interaction (concentration × site × dosage) has a mean square (72.59), which is the largest of any shown in Table 2. Although this interaction is not statistically significant, it does indicate a tendency for nonreturns to be highest when the larger amounts of the more highly concentrated semen are deposited in the horns, the body, and the cervix simultaneously.

On the average over the four sites of deposition, there was an increase of 1.2% in nonreturns for each 10 million increase in total sperm deposited (total sperm = concentration × dosage). This effect was not quite significant at the 5% level. The total number of spermatozoa deposited varied from 4 to 48 million. It appeared that fertility declined about 3.3 percentage units when the number of spermatozoa per insemination was decreased from 12 million to 8 million. There was apparently no difference in the fertility obtained by depositing 4, 6, or 8 million spermatozoa.

SUMMARY

Inseminators in 16 artificial breeding locals participated for 4 months in a study of the effect on fertility of dosage, site of deposition, and concentration of spermatozoa in diluted semen.

Four sites of deposition were used: (a) half-way through the cervix, (b) body of the uterus, (c) both uterine horns, and (d) both uterine horns, body of the uterus, and the cervix. The four dosages used were 0.25 ml., 0.5 ml., 1.0 ml., and 2.0 ml. The two concentrations used were 16 million spermatozoa per milliliter and 24 million spermatozoa per milliliter.

A total of 9,558 cows were bred during the experimental period, or an average of 298.7 cows for each combination of site, dosage, and concentration used. There were no statistically significant differences in the fertility obtained by the various methods of insemination. However, there was a tendency for the higher concentrations, larger dosages, and deeper depositions to produce the highest fertility, with an average increase of 1.2% in nonreturns for each 10 million additional sperm deposited. The total number of spermatozoa deposited varied from 4 to 48 million. It appeared that fertility declined about 3.3 percentage units when the number of spermatozoa per insemination was decreased from 12 million to 8 million. There was apparently no difference in the fertility obtained by depositing 4, 6 or 8 million spermatozoa per insemination.

REFERENCES

(1) Branton, C., Newsom, M. H., and Patrick, T. E. Diluting Bull Semen on the Basis of Numbers of Spermatozoa Rather than by Volume. (Abs.) J. Dairy Sci., **32**: 721-722. 1949.

(2) Donald, H. P. Heat During Pregnancy in Dairy Cows. Vet. Record, **55**: 297-298. 1943.

(3) Holt, A. F. Comparison Between Intracervical and Intrauterine Methods of Artificial Insemination. Vet. Record, **58**: 309-310. 1946.

(4) Knight, C. W., Patrick, T. E., Anderson, H. W., and Branton, C. The Relation of Site of Semen Deposit to Breeding Efficiency of Dairy Cattle. J. Dairy Sci., **34**: 199-202. 1951.

(5) Lasley, J. F., and Bogart, R. Some Factors Influencing Reproductive Efficiency of Range Cattle Under Artificial and Natural Breeding Conditions. Mo. Agr. Expt. Sta. Research Bull. 376. 1943.

(6) Raps, G. Relative Effectiveness of Two Methods Used in Insemination of Dairy Cattle. North Am. Veterinarian, **29**: 221-222. 1948.

(7) Salisbury, G. W., Beck, G. H., Elliott, I., and Willett, E. L. Rapid Methods for Estimating the Number of Spermatozoa in Bull Semen. J. Dairy Sci., **26**: 69-78. 1943.

(8) Salisbury, G. W., and Bratton, R. W. Fertility Level of Bull Semen Diluted at 1:400 with and without Sulfanilamide. J. Dairy Sci., **31**: 817-822. 1948.

(9) Salisbury, G. W., and VanDemark, N. L. The Effect of Cervical, Uterine and Cornual Insemination on Fertility of the Dairy Cow. J. Dairy Sci., **34**: 68-74. 1951.

(10) Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press. Ames. 1946.

(11) VanDemark, N. L., Salisbury, G. W., and Boley, L. E. Pregnancy Interruption and Breeding Techniques in the Artificial Insemination of Cows. J. Dairy Sci., **35**: 219-223. 1952.

(12) Weeth, H. J., and Herman, H. A. Comparative Efficiency of Intracervical and Intrauterine Methods of Insemination in Dairy Cattle. J. Dairy Sci., **34**: 195-198. 1951.

(13) Willett, E. L. Fertility and Livability of Bull Semen Diluted at Various Levels to 1:300. J. Dairy Sci., **33**: 43-49. 1950.

(14) Willett, E. L., and Buckner, P. J. The Determination of Numbers of Spermatozoa in Bull Semen by Measurement of Light Transmission. J. Animal Sci., **10**: 219-225. 1951.

CHANGES IN RESPIRATION AND HEART RATES, BODY TEMPERATURES, PLASMA LACTIC ACID LEVELS AND PLASMA CREATININE LEVELS CAUSED BY STRESS IN DAIRY CATTLE [1, 2]

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Conceivably, one of the factors involved in milk production is the ability of the animal to adjust itself to a changing environment, and differences in levels of production between animals can in part be attributable to differences in abilities to make environmental adjustment. It is also to be expected that with a fuller knowledge of the effect of environmental factors upon levels of production more favorable conditions can be provided. This report deals with observations on the effects of low ambient temperature, exercise, intermittent electrical shock, adrenaline injections, dehorning, and parturition upon rectal temperature, respiration and heart rates, and plasma lactic acid and creatinine levels.

Gaalaas (9), Kibler and Brody (11), Rhoad (17), and Seath and Miller (19) have reported increased respiration rates with increases in environmental temperatures. Kelly and Rupel (10) and Seath and Miller (18) noted increased respiration rates with increases in relative humidity. Kibler and Brody (11) noted a progressive decrease in respiration rate of 10 to 15 per minute when temperatures were gradually dropped from 50 to 5° F. Regan and Richardson (16) also observed decreased respiration rates with lowered temperatures.

Kelly and Rupel (10), Kibler and Brody (11), and Regan and Richardson (16) observed decreases, whereas Blaxter and Price (5) and Seath and Miller (18) observed increases in pulse rates with increasing environmental temperatures. Kibler and Brody (11) also reported that as temperatures were gradually decreased from 50 to 5° F. there was an increase of approximately 8% in pulse rate.

No changes in rectal temperatures were observed with decreases in environmental temperature in reports by Kibler and Brody (11) and Regan and Richardson (16).

Blaxter and Price (5) reported a 15 to 40% rise in heart rate and 0.75 to 1.25° F. rise in rectal temperature during the last 12 weeks of pregnancy. Fuller (8) observed pulse rates of 76 for high and 68.6 for moderate producing cows with respiration rates of 35 and 28.6, respectively.

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[1] Scientific Journal Series Paper No. 2982, Minnesota Agricultural Experiment Station.

[2] The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, University of Minnesota, in partial fulfillment of the requirements for the Ph.D. degree, August 1951.

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Respiration and pulse rates as well as body temperatures increased, according to Minett (13), when normal cows were exercised for one hour on level ground. It required 30 minutes for respiration rates, 40 minutes for pulse rates, and 50 minutes for body temperatures to return to normal after exercise. Woodward (21) reported an increase in the fat percentage of the milk when cows were walked three miles. Slight excitation increased the pulse rate 5 to 6 beats per minute, according to Blaxter (4), who also reported that standing, ruminating, and eating caused increased heart rates. Heart rate decreases with advance in age, according to Alfredson and Sykes (1) and Dukes (6).

According to Best and Taylor (3), adrenaline injection slows but strengthens heart action. Elevated blood pressure is responsible for reducing the heart rate. Amounts of adrenaline too small to increase blood pressure may cause a rise in blood sugar. At first there is usually a short period of apnea followed by an increase in the rate and depth of respiration. Elevation in blood pressure was found by Milks (12) to be due to the constricting effect of adrenaline upon the blood vessels.

EXPERIMENTAL PROCEDURE

Thirty animals were used in the experiment. The animals were members of 12 sets of identical twins and 2 sets of identical triplets whose ages ranged from 1 to 3½ years. In all the experiments, with the exception of the series dealing with dehorning, each member of a set of twins was used as a control for its mate while the other was subjected to periods of stress. Before an animal was subjected to the experimental treatment a preexperimental observation was made on the animal.

The effects of stress on the animals were determined by changes in rectal temperature, respiration and heart rates, and plasma lactic acid and creatinine levels. The lactic acid content of the blood and plasma was determined by the method of Barker and Summerson (2) and the plasma creatinine levels by the method of Folin and Wu (7). A standard veterinary thermometer was inserted at least 4 in. into the rectum and allowed to remain for approximately 2 minutes to obtain the rectal temperature. Respiration and heart rates were obtained by the use of a stethoscope.

All the data were analyzed statistically. The effects of the various stress conditions were tested for significance by analysis of variance as outlined by Snedecor (20). The "least significant difference" was determined on the data pertaining to the effect of adrenaline on the heart rate and the effect of parturition on the various measures of stress studied.

RESULTS AND DISCUSSION

The effect of low ambient temperature. Four sets of identical twin heifers were used to determine the effect of cold environmental temperatures on dairy cattle. One member of the twin set was used as a control and the other as the experimental animal. Five observations were made to determine the effect of the cold environmental air on the animals. The animals were taken from the

barn with a temperature of 45° F. and exposed to outside environmental temperatures from +16 to —16° F. One animal was subjected to a temperature of —16°, one to —7°, one to —2°, one to +1° and a fifth one to +16° F. The control animals were observed in the barn at a temperature of 45° F.

Respiration and heart rates and rectal temperatures were observed on both the control and the experimental animals prior to the experimental period and immediately after the animals had been in the cold air for 1 hour. In three cases observations were taken 1 hour after the animal was returned to the warm barn. The results are summarized in Table 1.

TABLE 1

The effect of low ambient temperature on the respiration and heart rates, rectal temperatures, and blood lactic acid levels (5 animals in each group)

Condition	Periods [a]		
	A	B	C
		Respiration rates *(No./min.)*	
Control	21	22	21
—16° to +16° F.	21	20	23
		Heart rates *(No./min.)*	
Control	100	96	101
—16° to +16° F.	97	109	103
		Rectal temperatures *(° F.)*	
Control	101.9	101.6	101.6
—16° to +16° F.	101.8	102.4 [b]	101.9
		Blood lactic acid *(mg. %)*	
Control	13.0	12.5	12.1
—16° to +16° F.	14.3	19.5 [c]	11.0
Observations, No.	5	5	3

[a] Period A — Observation before being placed in low ambient temperature.
B — Immediately after cold treatment.
C — One hour after cold treatment.
[b] Differences statistically significant.
[c] Differences statistically highly significant.

The lactic acid content of the blood rose from a level of 14.3 mg. % before exposure to 19.5 mg. % after 1 hour of exposure to —16 to +16° F. environmental temperatures and subsided to 11.0 mg. % 1 hour after the end of the exposure period. It was observed that in every instance the lactic acid content of the blood increases during exposure. The average increase for all animals was 36.4%. One animal (T 52) showed only a slight increase in lactic acid level of the blood after being subjected to low temperature. This fact is not surprising, as this animal had a very high lactic acid level before she was placed in the cold air. The rather high value possibly could have been caused by excitement or apprehension due to the fact that she was able to observe her

stablemate being subjected to having a blood sample taken. The changes in lactic acid caused by exposure to low temperature were highly significant.

There was an increase in the rectal temperature from 101.8 to 102.4° F. after the animals were subjected to the cold air. One hour after exposure the temperature had returned to 101.9° F. Each animal exhibited an increase in rectal temperature after exposure to low environmental temperatures for a period of 1 hour. This increase was from 0.1 to 1.2° F. in the five observations and was significant.

The effect of low environmental temperatures on the heart rate was shown by a rather pronounced increase at the end of a 1-hour period of exposure by four of the animals. For all of the animals this increase amounted to 12.4%. There was no increase in the heart rate of one animal after exposure. The heart rates of the control animals were subject to considerable variation.

The changes in respiration rates were not consistent during the experiment. In two instances there was a decrease; in two, an increase; and no change in the fifth.

All of the animals showed some evidence of discomfort when taken out of the barn and placed in the cold air. They tended to shiver, humped their backs, and became quite active. It is probable that the increased muscular activity contributed largely to the increase in the lactic acid levels of the blood. Kibler and Brody (11) reported an increase in heat production when dairy animals were placed in an environment in which the temperature was gradually decreased from 50 to 5° F. They observed no significant changes in rectal temperatures. This probably was due to lack of shivering and less need for the body to rapidly mobilize the heat retention or heat production mechanism, since the temperature was lowered gradually.

The increase in heart rate of 12.4% agrees reasonably well with the work of Kibler and Brody (11), who found an increase of approximately 8%. They also reported approximately 50% decrease in respiration rates when the temperature was gradually reduced to 5° F. In the work being reported only one value approached such a figure, that of animal T 52, whose respiration rate decreased from 28 to 20 per minute. The respiration rates reported here are in agreement with the findings of Gaalaas (9), Kelly and Rupel (10), and Regan and Richardson (16).

The effect of exercise. Normally, when not confined, the dairy animal moves about to a greater or less extent. To study the effect of exercise, five sets of identical twin dairy heifers ranging in age from 12 to 18 months were used. The quantity of exercise each obtained was a variable factor and consisted of forced running for 10 minutes up and down a roadway approximately 300 feet long. The amount of exercise depended somewhat on the willingness of the animal to cooperate. Observation of respiration and heart rates and rectal temperatures was made just prior to the exercise period and afterward by three other observations: one immediately after exercise, another 30 minutes after, and the last one 90 minutes after exercise. Blood samples were also taken to determine whether or not exercise affects the plasma lactic acid and creatinine levels. The results are summarized in Table 2.

TABLE 2

The effect of exercise on the respiration and heart rates, rectal temperatures, and plasma lactic acid and creatinine levels (10 animals in each group)

Condition	Periods [a]			
	A	B	C	D
		Respiration rates (*No./min.*)		
Control	24	23	24	26
Exercise	24	37 [b]	29	26
		Heart rates (*No./min.*)		
Control	78	78	78	78
Exercise	78	131 [c]	86	77
		Rectal temperature (*° F.*)		
Control	101.9	101.8	101.7	101.9
Exercise	101.7	102.0 [b]	102.1	101.9
		Plasma lactic acid (*mg. %*)		
Control	12.2	11.2	9.4	9.2
Exercise	11.0	41.0 [c]	17.9	10.3
		Plasma creatinine (*mg. %*)		
Control	1.07	1.02	1.01	1.05
Exercise	1.01	1.13	1.04	1.03
Observations, No.	10	10	10	10

[a] Period A — Before exercise.
B — Immediately after exercise.
C — Thirty minutes after exercise.
D — Ninety minutes after exercise.
[b] Statistically significant.
[c] Statistically highly significant.

Exercise proved to be instrumental in causing highly significant changes in respiration and heart rates and lactic acid levels of the blood plasma and a significant change in rectal temperatures. The respiration rate was increased in every instance by exercise and averaged 54.2%. One-half hour after the cessation of exercise, the respiration rate was still slightly elevated, but at the end of 90 minutes it had reached normal levels. A most dramatic effect produced by the exercise was on the heart rate. This showed an increase of 67.9%, rising from 78 to 131 beats per minute. The high rate subsided to 86 per minute 30 minutes after exercise and to a normal 77 per minute 90 minutes after exercise. The control animals showed a remarkably constant average of 78 heart beats per minute for all four observations. The differences between heart rates of the experimental animals immediately after exercise and of the control animals was highly significant.

The effects of exercise on the rectal temperature and lactic acid and creatinine plasma levels also are shown in Table 2. The effect on the rectal temperature was not uniform for all of the animals. The average increase was slight, 101.7 to 102.0° F., when observed immediately after the exercise. The

temperatures observed 30 minutes after exercise followed no apparent trend but showed a further increase when all the individual observations were averaged.

There was a very great increase in the plasma lactic acid levels as a result of exercise. The smallest increase amounted to 94.9%, and the greatest increase was 420.0%. The average increase for all of the animals was 272.7% when observed immediately after exercise. Thirty minutes after exercise the plasma lactic acid levels were still above preexercise levels. The control animals exhibited very little fluctuation from one period to the other. The average lactic acid levels of the four observations fluctuated only a total of 3 mg. %, the minimum being 9.2 and the maximum 12.2 mg. %.

The creatinine levels of the plasma of the animals after exercise did not differ significantly from the levels before exercise. The average level of creatinine before exercise for all animals was 1.01 mg. %. This rose to 1.13 mg. % after exercise and approached normal values 30 minutes after the cessation of exercise. The plasma creatinine levels in only one animal did not increase following exercise. The average level for the control animals remained relatively constant.

It is recognized that exercise has a profound effect upon the cardiorespiratory processes and upon certain constituents of the blood. Mullick (14) found that the so-called "panters" possessed higher lactic acid levels in the blood than did the control animals. This stress condition was a factor, therefore, in the increased lactic acid level and is similar to the results presented here where the lactic acid content rose to very high levels immediately after exercise. Woodward (21), in studying the factors that influence the percentage of fat in milk, found that exercise improved the quality of milk produced, indicating that exercise may affect other body processes also.

Palmer *et al.* (15) also demonstrated that exercise affects the composition of the blood plasma. They reported a rise of 6.34% in the inorganic phosphorus content of the blood plasma immediately after exercise, which was followed by a drop of 22% 20 minutes later.

The effect of intermittent electrical stimuli on dairy cattle. Five sets of twins were used as experimental animals in studying the effect of intermittent electrical stimuli applied by the use of a commercial electrical prod commonly used by livestock truckers and livestock men.

The prod was placed on the animal's thigh and the electrical stimuli, with a frequency of 60 per minute, applied for about 30 seconds. The reactions of the individual animals varied considerably in spite of floor insulation. The variation observed may have been due to the thickness of the hair coat causing variation in contact between the prod and the hide of the animal. The animals were restrained in stanchions for all observations.

Regardless of the number of stimuli, the respiration rate showed increase when taken immediately after treatment. This increase ranged from 3 to 17 per minute. The results are shown in Table 3. The increased rate prevailed even an hour later in a few cases. This persistency of the elevated respiration rates was due probably to the fact that the animals were apprehensive of the

person taking the respiration rates an hour later. For the experimental animals, the average respiration rate prior to the treatment was 25 per minute, and immediately after treatment the average rate rose to 36, or an increase of 44%. The rate after 1 hour was 12% above the rate prior to treatment.

The increase in respiration rates of the experimental animals was highly significant when compared to the respiration rates of the control animals immediately after electrical stimuli were applied. The rates of the control and experimental animals were not significantly different when observed 1 hour later.

TABLE 3

The effect of intermittent electrical stimuli on the respiration and heart rates, rectal temperatures and plasma lactic acid and creatinine levels (10 animals in each group)

Condition	Periods [a]			
	A	B	C	D
		Respiration rates *(No./min.)*		
Control	23	24	25	24
Shocked	25	36[b]	28	24
		Heart rates *(No./min.)*		
Control	80	80	79	78
Shocked	81	129[b]	88	84
		Rectal temperature *(° F.)*		
Control	101.7	101.9	101.9	101.8
Shocked	101.7	101.9	102.1	102.2
		Plasma lactic acid *(mg. %)*		
Control	9.3	10.2	8.8	5.2
Shocked	9.0	21.7[b]	9.4	6.1
		Plasma creatinine *(mg. %)*		
Control	1.07	1.09	1.13	1.06
Shocked	1.07	1.10	1.11	1.12
Observations, No.	10	10	10	10

[a] Period A — Before stimulation.
B — Immediately after stimulation.
C — Thirty minutes after stimulation.
D — Ninety minutes after stimulation.

[b] Differences statistically highly significant.

The effect of excitation on the heart rate was more dramatic and pronounced. The average heart rate for all the animals subjected to the shock treatment was 81 before treatment, 129 immediately after treatment, and 88 an hour later. The variations were from 32 to 87% higher than the rates of the period prior to shock. A high of 159 was observed for both members of one set of twins. There were some carry-over effects 1 hour later, as would be expected, but they were not pronounced. The change in heart rate caused by the electrical shock was highly significant.

There were no changes observed in the rectal temperatures of the dairy cattle following intermittent electrical stimuli. The effect on lactic acid levels of the plasma showed considerable variation between animals from little or no change to marked elevation. The average increase in plasma lactic acid levels immediately after treatment for all animals was 141%. The number of times the stimulus was applied appeared to have no relationship to the changes in the lactic acid levels of the plasma of the animals. The levels were all back to normal 1 hour after treatment. The plasma creatinine levels, shown in Table 3, were not appreciably affected immediately after the electrical stimuli were applied.

The effect of dehorning on dairy cattle. During the time that data were being collected, one set of twins and one set of triplets were dehorned after anesthesia [4] was established in the horn area. Each triplet also had a ring placed in its nose. Observations were made just prior to, immediately after, and 5 to 6 hours after, dehorning. The results of the observations are presented in Table 4.

TABLE 4

The effect of dehorning on the respiration and heart rates, rectal temperatures and the blood lactic acid levels (Average for 5 animals)

Measure of stress	Periods[a]			No. of observations
	A	B	C	
Respiration rate *(No./min.)*	21	17	17	5
Heart rate *(No./min.)*	76	107[b]	106	5
Rectal temperature *(° F.)*	101.4	101.4	101.6	5
Lactic acid levels *(mg. %)*	14.7	19.4	14.1	5

[a] A — Before dehorning.
B — Immediately after dehorning.
C — Five to six hours after dehorning.
[b] Differences statistically significant.

Heart rates, which increased from an average of 76 before to 107 beats per minute after the operation, were significantly changed as a result of dehorning. The rectal temperatures were unaffected, and the average increase of 4.7 mg. % in plasma lactic acid levels is not significant. It must be noted that although the data for all animals show no significant change except for heart rate, which remained high for 5 to 6 hours after the operation, respiration rates for the triplets were depressed after dehorning.

The effect of adrenaline injection. Five sets of Grade Guernsey, female identical twins, ranging in age from 11 to 18 months, were used in this series. Each set of twins was used as a separate experiment in which one twin was used as the control and the other as the experimental animal. Later, the experiment was repeated on the same set of twins; however, the twin used as a control in the first experiment became the experimental animal. In this way each twin of a set was subjected to the same treatment.

[4] Approximately 10 ml. of a 1% solution of Procaine was used at the base of each horn.

The rectal temperature, respiration rate, and plasma lactic acid and heart rate were noted before and immediately after, 30 minutes after, and 60 minutes after, intrajugular administration of 1½ to 2 ml. of adrenaline.[5] The results of these observations are presented in Tables 5 and 6.

TABLE 5

The effect of adrenaline on the respiration rates, rectal temperatures, and plasma lactic acid levels on five sets of identical twins (10 animals in each group)

Condition	Periods [a]			
	A	B	C	D
		Respiration rates (*No./min.*)		
Control	29	28	29	29
Adrenaline	27	26[b]	26	26
		Rectal temperature (*° F.*)		
Control	101.9	101.9	101.8	101.9
Adrenaline	101.7	101.8	102.0	102.1
		Plasma lactic acid (*mg. %*)		
Control	8.2	9.4	8.8	8.2
Adrenaline	8.0	29.2[c]	20.9	11.4
Observations, No.	10	10	10	10

[a] Period A — Before adrenaline injection.
B — Immediately after injection.
C — Thirty minutes after injection.
D — Sixty minutes after injection.
[b] Differences statistically significant.
[c] Differences statistically highly significant.

TABLE 6

The effect of adrenaline on the heart rates of three animals before, and 1 to 45 minutes after, injection[a]

Animal No.	Time after injection	Heart rate	Animal No.	Time after injection	Heart rate
		(*No./min.*)			(*No./min.*)
T 54	Before	74	T 61	Before	87
	1 min.	76		1 min.	47
	5 min.	68		3 min.	57
	10 min.	78		5 min.	77
T 57	Before	77	T 62	Before	60
	1 min.	58		2 min.	42
	5 min.	68		4 min.	44
	10 min.	67		15 min.	70
				45 min.	60
T 58	Before	88			
	1 min.	52			
	5 min.	77			
	10 min.	87			

[a] Taken from electrocardiographic recording.

[5] The adrenaline used was a product manufactured and produced by Armour Laboratories, Chicago, Ill., under the trade name, Suprenalin. Solution 1:1000 (Epinephrine Hydrochloride) containing 0.1% epinephrine per cc.

Observations were made also on the general attitude of the animal and its reaction to the adrenaline injection. Particular attention was directed to posture, muscle tremors, perspiration, erection of hair, urination, depth of respiration, evidence of bodily discomfort, salivation, and lacrymation.

In every instance there was an increase in the lactic acid levels of the blood plasma. The average increase was 29.2 mg. % for the ten animals. This represented an increase of 265.0% when compared with the control period and was highly significant. There was great variation in the lactic acid levels of the individual animals after adrenaline injection. The greatest increase, 546.4%, was shown by T 58 and the smallest increase, 86.5%, by T 60. Rather high lactic acid levels in the plasma were still apparent 30 minutes after adrenaline injection, but in 90 minutes the levels of lactic acid were rapidly approaching the preexperimental levels.

The quantity of adrenaline injected, at least in the ranges used, appeared to have no uniform effect on the lactic acid levels of the blood plasma. Immediately after adrenaline injection there was a sharp increase in the lactic acid, followed by a sharp decrease to about one-half of the level between the control and the level immediately after injection. At the end of 90 minutes after injection, the plasma levels were only slightly above the control levels.

The rectal temperature changes showed little uniformity with regard to the effect of adrenaline injections. There appeared to be a tendency for the temperature to increase slightly. In only one animal in this series (T 58) was

TABLE 7

The effect of adrenaline on heart rates of three animals before, and 15 to 30 seconds after, injection[a]

Animal No.	Time after injection	Heart rate	Animal No.	Time after injection	Heart rate
		(No./min.)			*(No./min.)*
T 51	Before	86	T 59	Before	76
	15 sec.	86		15 sec.	125
	30 sec.	41		30 sec.	90
	60 sec.	48		40 sec.	obscure
	2 min.	52		1 min.	obscure
	3 min.	64		2 min.	obscure
	10 min.	70		7 min.	75
				10 min.	76
				30 min.	86
T 52	Before	77	T 60	Before	90
	15 sec.	100		15 sec.	125
	30 sec.	65		30 sec.	70
	45 sec.	77		1 min.	45
	5 min.	74		3 min.	49
				5 min.	55
T 53	Before	80			
	15 sec.	95			
	35 sec.	obscure			
	5 min.	47			
	10 min.	57			

[a] Taken from continuous electrocardiographic recording.

there a definite increase evident. The number of respirations decreased slightly after adrenaline administration.

In every instance there was a decrease in the heart rate, as shown in Tables 6 and 7. It is also evident from the data presented in Table 7 that four of the five animals in this part of the experiment showed a definite increase in heart rate before a decrease resulted. This increase was as high as 64.4% in one instance.

The decrease in heart rate observed 1 minute after adrenaline injection was significantly different from the control heart rate. The observations made 5 minutes after treatment were not significantly different.

The effect of parturition on dairy cattle. To study the effects of parturition on dairy cattle, four sets of twins and one set of triplets were used. One animal of each of the twin sets and two animals of the triplet set were used for controls, and the other members were used as experimental animals.

In only one twin set was it possible to use both animals as experimental subjects. All three members of the triplet set calved and consequently could be used to determine the effect of parturition.

There were increases in respiration and heart rates, temperatures, and plasma lactic acid levels on the day of calving, the greatest increases occurring immediately after calving. The animals quickly returned to normal and by the day following calving were essentially back to normal levels. It was observed further that even though there appeared to be an average increase in respiration with parturition, this was not uniformly shown by all animals. In only three cases was there definite evidence that there was an increase. The results are summarized in Table 8.

The respiration rates rose from 32 per minute to 55 per minute 2 hours after calving and returned to normal on the day following calving. This represents an increase of approximately 68.8%.

The heart rate followed a rather definite pattern for all groups. As parturition approached, the heart rate increased. After parturition there was a greater increase, and it was a day or two after the day of parturition before the rates returned to normal. The heart rate increased from 82 to 97 per minute on the day of parturition. This represents an increase of 17.2% over the preexperimental period. However, compared to the heart rate of the control animals during this same period, the experimental animals exhibited an increase of 29%.

Rectal temperatures were variable for these trials. Temperatures tended to rise 2 days before parturition, then subside the day before, and rise sharply on the day of parturition. There was again a tendency for the temperature to decline the next day.

The plasma lactic acid levels increased on the day of calving and quickly subsided to normal the day following parturition. There is considerable muscular activity during parturition, and with this increased muscular activity an increase of lactic acid in the plasma level is expected. The lactic acid levels rose to 17.5 mg. % immediately after parturition, or 194.2% above the control animals during the same period. For the most part the normal levels of plasma lactic acid were from 6 to 7 mg. % and the extremes were from 5.9 to 8.2 mg. %.

TABLE 8

The effects of parturition on respiration rates, heart rates, rectal temperature and plasma lactic acid (10 control and 7 experimental animals)

Condition	Days before calving			Day of calving				Days after calving		
					Time after calving					
	3	2	1	Before calving	Immediately	2 hr.	3 hr.	1	2	3
				Respiration rates (*No./min.*)						
Control	34	31	35	27	41	47	45	33	32	34
Parturition	33	32	32	36	43	55	55	33	37	42
				Heart rates (*No./min.*)						
Control	74	74	74	70	75	72	76	74	75	72
Parturition	80	79	82	86	97	85	79	78	76	72
				Rectal temperature (*° F.*)						
Control	102.0	102.1	102.1	101.9	101.9	102.1	102.2	102.1	101.8	102.0
Parturition	102.6	102.4	101.7	101.6	102.9	103.0	102.6	101.5	101.6	101.8
				Plasma lactic acid (*mg. %*)						
Control	7.4	6.4	8.2	6.3	5.9	5.9	6.9	6.0	7.0	8.0
Parturition	7.1	6.5	7.5	8.8	17.5	11.9	10.6	7.4	6.6	7.3
				Observations (*No.*)						
Control	10	10	10	6	10	8	6	10	10	8
Parturition	7	7	7	5	7	6	4	7	7	6

SUMMARY AND CONCLUSIONS

The effect of low environmental temperature, exercise, dehorning, adrenaline administration, parturition, and intermittent electrical stimuli on dairy cattle was measured by determining rectal temperatures, respiration and heart rates, and lactic acid and creatinine levels in the blood.

When animals were placed in a low environmental temperature of from —16 to +16° F. the heart rates increased 12.4% and the respiration rates showed little change. There was a significant increase (0.8° F.) in rectal temperatures and a highly significant increase (36.4%) in the blood lactic acid levels.

When the animals were dehorned, there were no significant changes observed in respiration rates, rectal temperatures, or blood lactic acid levels. There was a significant rise in the heart rate, which increased 40.8%.

Vigorous exercise for 10 minutes caused highly significant increases in respiration rates (54.2%), heart rates (67.9%), and plasma lactic acid levels (272.7%). A significant rise of rectal temperature also resulted.

The introduction of 1-2 ml. of adrenaline (1:1000) into the blood stream via the jugular vein caused a highly significant rise in plasma lactic acid levels and also an increase in heart rate 15 seconds after injection, followed by a decrease 60 seconds after injection. A significant decrease was observed also in the respiration rates.

Intermittent electrical stimuli produced highly significant increases in respiration rates and plasma lactic acid levels. A significant increase in heart rates but no appreciable changes in rectal temperatures and plasma creatinine levels were observed.

Parturition produced no significant changes in the factors studied.

REFERENCES

(1) Alfredson, B. V., and Sykes, J. F. Electrocardiograph Studies in Normal Dairy Cattle. J. Agr. Research, **65**: 61-87. 1942.

(2) Barker, S. B., and Summerson, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. J. Biol. Chem., **138**: 535-554. 1941.

(3) Best, C. H., and Taylor, N. B. *The Physiological Basis of Medical Practice.* 5th ed. The Williams and Wilkins Co., Baltimore. 1950.

(4) Blaxter, K. L. The Normal Variation in the Heart Rate of Dairy Cattle. Vet. J., **99**: 2. 1943.

(5) Blaxter, K. L., and Price, H. Variations in Some Physiological Activities of Dairy Cows. Vet. J., **101**: 39. 1945.

(6) Dukes, H. H. *The Physiology of Domestic Animals.* 6th ed. Comstock Publishing Co., Inc., Ithaca, N. Y. 1947.

(7) Folin, O., and Wu, A. A System of of Blood Analysis. J. Biol. Chem., **38**: 81-110. 1919.

(8) Fuller, J. M. Some Physical and Physiological Activities of Dairy Cows. N. H. Agr. Expt. Sta. Tech. Bull. 35. 1928.

(9) Gaalaas, R. F. Effect of Atmospheric Temperature on Body Temperature and Respiration Rate of Jersey Cattle. J. Dairy Sci., **28**: 555-563. 1945.

(10) Kelly, M. A. R., and Rupel, I. W. Relation of Stable Environment to Milk Production. U.S.D.A. Tech. Bull. 591. 1937.

(11) Kibler, H. H., and Brody, S. Influence of Temperature, 50° to 5° F. and 50° to 95° F. on Heat Production and Cardiorespiratory Activities of Dairy Cattle. Mo. Agr. Expt. Sta. Research Bull. 450. 1949.

(12) Milks, H. J. Uses of Some Drugs Acting Upon the Circulatory System. Cornell Vet., **23**: 152-160. 1933.

(13) Minett, F. C. Panting in Cattle. A Sequel to Foot and Mouth Disease. I. Experimental Observations and Pathology. Am. J. Vet. Research, **10**: 40-48. 1949.

(14) Mullick, D. N. Panting in Cattle. A Sequel to Foot and Mouth Disease. II. Bio-Chemical Observations. Am. J. Vet. Research, **19**: 49-55. 1949.

(15) Palmer, L. S., Cunningham, W. S., and Eckles, C. H. Normal Variations in the Inorganic Phosphorous of the Blood of Dairy Cattle. J. Dairy Sci., **13**: 174-195. 1930.

(16) Regan, W. M., and Richardson, G. A. Reactions of the Dairy Cow to Changes in Environmental Temperature. J. Dairy Sci., **21**: 73-79. 1938.

(17) Rhoad, A. O. The Influence of Environmental Temperature on the Respiratory Rhythm of Dairy Cattle in the Tropics. J. Agr. Sci., **26**: 36-44. 1936.

(18) Seath, D. M., and Miller, G. D. The Relative Importance of High Temperature and High Humidity as Factors Influencing Respiration Rate, Body Temperature, and Pulse Rate of Dairy Cows. J. Dairy Sci., **29**: 465-472. 1946.

(19) Seath, D. M., and Miller, G. D. Repeatability of Heat Tolerance Observations. J. Dairy Sci., **30**: 957-962. 1947.

(20) Snedecor, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. 1946.

(21) Woodward, T. E. Factors Influencing Percentage of Fat in Milk. J. Dairy Sci., **6**: 466-478, 1923.

THE EFFECT OF THYROPROTEIN FEEDING ON THE BLOOD LEVEL OF INORGANIC IODINE, PROTEIN-BOUND IODINE AND CHOLESTEROL IN DAIRY COWS

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Through the recent development of both thyroid active and thyroid inhibiting substances, it is now possible to maintain animals at almost any level of thyroid activity that may be desired. It has been shown that by feeding thyroprotein, an iodinated casein preparation, to dairy cows it is possible to increase milk and butterfat production (16). The use of thyroidal materials and synthetic goitrogens in controlling thyroid status in animal production has been extensively reviewed by Blaxter *et al.* (1).

The possibilities for practical application of these materials to thyroid regulation in animal production has focused attention on the need for a simple laboratory test which can be used as an indicator of thyroid activity and thus serve as a guide to the most effective use of these materials.

Two constituents of the blood are known to be influenced by thyroid activity, namely, protein-bound iodine (PBI) and cholesterol. The blood protein-bound iodine determination under usual conditions is a measure of the amount of thyroid hormone circulating in the blood and has been used clinically as an indicator of thyroid activity (14). Blood cholesterol has been known since 1922 to have an inverse relationship to metabolic rate, as the result of the work of Epstein and Lande (4). Since that time blood cholesterol has been used rather widely as an indirect clinical test for thyroid activity (13).

It was shown previously (8) that feeding thyroprotein to cows resulted in a decrease in blood cholesterol followed by an increase to levels above normal after thyroprotein feeding was terminated. The blood cholesterol was found to decrease to approximately 40% of the normal level in parturient cows fed thyroprotein compared to a decrease of about 20% at parturition when no thyroprotein was fed.

In previous reports (7, 9, 10, 11, 15) some of the normal variations in cattle associated with age, inheritance, rate of gain, and efficiency of feed utilization were reported along with a discussion of other implications of the test as it applies to functional response.

The objective in this study was to determine the changes occurring in blood serum PBI and blood plasma cholesterol during and after the feeding of thyroprotein [2] to dairy cows. It was desired to study the respective sensitiv-

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[2] Standardized "Protamone" (3% thyroxine) supplied by Cerophyl Laboratories, Inc., Kansas City, Mo.

ity of these two blood constituents at various levels of thyroid activity to gain some insight as to their accuracy and possible limitations as indicators of thyroid activity in cattle and to ascertain their possible relationship to each other under such conditions.

EXPERIMENTAL PROCEDURE

The inorganic and the protein-bound iodine levels in Experiment I were determined by the method of Connor *et al.* (2) using blood serum. These levels were found to be falsely high because of the large amounts of inorganic iodine in the thyroprotein which was not completely washed out. Accordingly, a modification was used in Experiment II involving four washings with distilled water instead of the usual one to prevent the inorganic iodine in the precipitate from contaminating the protein-bound fraction. Phosphorus acid, technical grade, was found to give less variability than the C.P. grade and accordingly was substituted in the procedure during Experiment II.

Blood plasma cholesterol determinations were made, using the modification of the Liebermann-Burchard reaction suggested by Saifer and Kammerer (18).

Two different experiments were conducted in which thyroprotein was fed. In Experiment I, three Ayrshire cows in their first lactation, each weighing approximately 1,000 lb., were fed thyroprotein at the rates of 10, 15, and 20 g. per day, respectively. Blood samples were drawn at 2 to 3 day intervals and analyzed for PBI and inorganic iodine. In Experiment II, two pairs of cows each consisting of one Jersey and one Holstein were fed thyroprotein at the respective rates of 15 and 25 g. daily per 1,000 lb. initial body weight over a period of 53 days. Blood samples were drawn at weekly intervals during the period of thyroprotein feeding and for 12 weeks thereafter. This series of blood samples was analyzed for PBI, inorganic iodine, cholesterol, and sugar.

A third experiment was conducted to determine the effect of the thyrotropic hormone on the levels of PBI and inorganic iodine.

RESULTS AND DISCUSSION

Experiment I. The effect of thyroprotein feeding on the serum inorganic and protein-bound iodine levels. Under normal feeding conditions, the inorganic iodine level remained within the range of 0.7 to 3.2 γ % (11). Blood inorganic iodine values in the cows fed thyroprotein were found to average approximately 50 times higher than in the controls during the thyroprotein feeding period. (Table 1.)

The normal range of PBI for cattle by this method was found previously to be 2.0-5.0 γ % (11). Among approximately 1,000 analyses performed to date in this laboratory no cow under normal feeding conditions has had a PBI higher than 10.9 γ %. In humans the normal range by this method was shown to be 3.0-6.0 γ %, and in the most severe cases of hyperthyroidism the highest levels reached were about 20 γ % (12).

The PBI values obtained for the cows fed thyroprotein rose to levels as high as ten times their own normal or that of the controls. This PBI level

was much higher than the clinical evidences of hyperthyroidism such as general appearance, pulse rate, respiratory rate, and body weight losses would indicate. A possible explanation is that the excessive amounts of inorganic iodine fed in the thyroprotein resulted in their combination with certain blood protein constituents to produce a large amount of noncalorigenic protein-bound iodine. A similar explanation has been offered for somewhat similar results obtained in human blood PBI analyses (14). Friedberg and Reineke (6) also have reported the presence of at least 10 iodinated compounds in thyroprotein, several of which could be removed by n-butanol extraction. Still further separation was

TABLE 1

The effect of thyroprotein feeding on blood serum inorganic iodine and protein-bound iodine. Experiment I

Date	Cow No. 365		Cow No. 373		Cow No. 371	
	10 g./day		15 g./day		20 g./day	
	Inorg. iodine	PBI	Inorg. iodine	PBI	Inorg. iodine	PBI
(1951)	(γ %)	(γ %)	(γ %)	(γ %)	(γ %)	(γ %)
4-11	1.7	3.5	1.3	3.7	1.7	4.1
4-12	1.7	3.9	1.7	3.7	1.7	3.8
4-17[a]	26.4	12.5	38.0	17.9	45.0	22.3
4-19	85.0	43.0	130.0	40.0	200.0	100.0
4-21	28.0	16.8	145.0	25.4	285.0	34.0
4-25	110.0	24.7	210.0	39.1	250.0	39.1
4-28[b]	30.0	9.2	125.0	12.1	125.0	13.3
4-30	200.0	25.9	325.0	25.9	380.0	47.0
5-3[c]	375.0	30.6	160.0	39.0	250.0	27.1
5-4	29.0	8.1	275.0	18.1	130.0	19.9
5-5	10.5	10.1	.....	.....	78.0	13.7
5-7	4.2	5.1	62.0	13.7	31.0	16.0
5-8	4.0	8.3	40.0	20.2	23.0	15.0
5-10	2.6	8.3	20.0	5.5	5.2	11.0
5-12	2.5	7.0	4.6	10.8	2.6	9.9
5-16	1.7	6.6	1.7	8.8	1.7	7.0

[a] Thyroprotein feeding started 4-16-51.
[b] No thyroprotein fed 4-28-51.
[c] Thyroprotein feeding terminated 5-3-51.

effected by an alkali wash. Reineke *et al.* (17) have shown further that the average thyroxine value obtained by the isotope dilution method was 22.9% that obtained by the n-butanol extraction method in samples of thyroprotein. The dependence of this type of protein-bound iodine on a continuous dietary intake of thyroprotein is suggested by the sudden drop after a feeding was missed on the 11th day of the feeding period and the rapid return to the high level after the resumption of feeding (Table 1). It is of interest to note, however, that in general the protein-bound iodine level in the blood varied according to the quantity of thyroprotein fed.

Experiment II. The effect of thyroprotein feeding on the serum inorganic and protein-bound iodine, plasma cholesterol and blood sugar levels. Thyroprotein was fed to two Jersey and two Holstein cows. One cow of each breed was fed, respectively, 15 and 25 g. daily per 1,000 lb. of initial body weight over a 53-day period. Blood samples were drawn at weekly intervals during

and after thyroprotein feeding and analyzed for serum inorganic and protein-bound iodine, plasma cholesterol, and blood sugar. The results are shown in Figures 1, 2, and 3. Blood sugar data are not shown because no differences were found to result from thyroprotein feeding.

In Figure 1 the average levels of inorganic iodine, protein-bound iodine, and blood cholesterol of all four cows (two Jerseys and two Holsteins) are shown during the thyroprotein feeding period, regardless of dosage. Two control cows are shown for comparison. The tremendous elevation of the inorganic

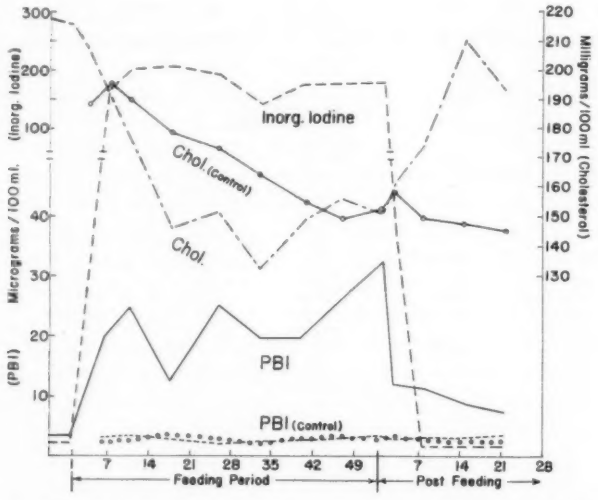

FIG. 1. The effect of feeding thyroprotein on the changes in serum inorganic iodine and protein-bound iodine (PBI) and plasma cholesterol of dairy cows. One Jersey and one Holstein at each dosage, 15 and 25 gms. / 1,000 lbs. / day combined. See Experiment II.

iodine during the feeding period is evident. The PBI level increased markedly as in Experiment I. The cholesterol level declined to a point below the control level after the beginning of thyroprotein feeding. The average cholesterol level during the first 3 weeks after the beginning of thyroprotein feeding was 33% below the control level. It had gradually returned to approximately the same level as that of the controls by the 53rd day, when the thyroprotein feeding was terminated. After thyroprotein feeding was terminated, the cholesterol rose to a level above the control and then gradually declined. Although the cholesterol level of the experimental cows returned to the control level before termination of thyroprotein feeding, whereas the PBI levels did not, the levels of these two substances after termination of thyroprotein feeding demonstrated the inverse relationship between thyroid activity and blood cholesterol. There was, however, no correlation between dosage and cholesterol level, nor were there any marked hereditary differences in the cholesterol levels due to breed.

A plausible explanation for the decrease in serum cholesterol in hyperthyroidism and the increase to levels above normal in hypothyroidism is offered by Hoffmann *et al.* as discussed by Foldes and Murphy (5). According to this hypothesis, lysolecithin, a toxic substance, is produced in hyperthyroidism in abnormally large amounts as a result of the presence of excess thyroxine. The organism attempts to counteract the toxic effects of lysolecithin by mobilizing its sterol reserves, mainly cholesterol. Toxic symptoms do not appear until the sterol reserves have been exhausted.

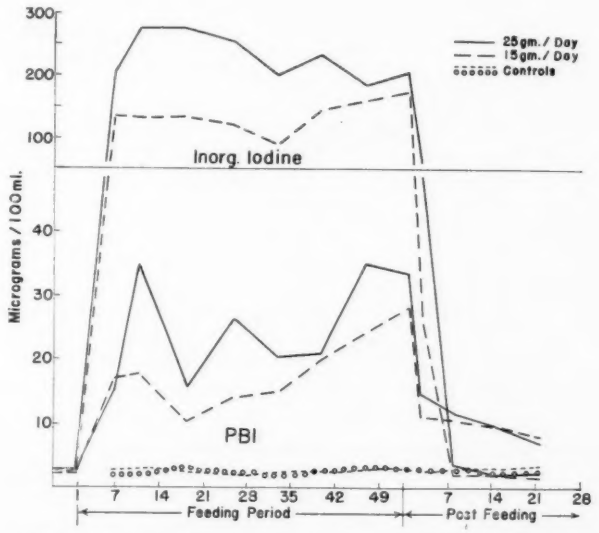

FIG. 2. The effect of feeding different amounts of thyroprotein (15 or 25 g. / day / 1,000 lb. of initial body weight) on the changes in serum inorganic iodine and protein-bound iodine of dairy cows. Two Jerseys and two Holsteins. See Experiment II.

This may, therefore, account for the low levels of cholesterol found after thyroprotein feeding was begun. The gradual return of the cholesterol toward normal even though the dosage remained constant may have, therefore, been due to an increased cholesterol synthesis or to the greater efficiency of mobilization of cholesterol reserves as the treatment continued. In other words, a physiological adjustment in cholesterol metabolism may have resulted. The return to a normal cholesterol level during thyroprotein feeding also may have been due in part to the reduction of thyroid activity of the cow's own thyroid gland due to the replacement effect of the thyroprotein, resulting in less thyroxine in the blood.

The sudden withdrawal of thyroprotein, leaving the cow in a temporary hypothyroid state, would have the result of less lysolecithin formation (5), reducing the breakdown of cholesterol as an antitoxin and thus causing an accumulation of cholesterol in the blood. This chain of events may explain the

rise in cholesterol to a point above the control level after the cessation of thyroprotein feeding. The subsequent return to normal would thus be due partly to the slowing up of cholesterol mobilization and partly to the return to normal of the animal's own thyroid mechanism in response to the physiological needs for thyroxine.

In Figure 2 the effect of feeding different quantities of thyroprotein daily, regardless of breed, is shown. It can be seen that throughout the feeding trial,

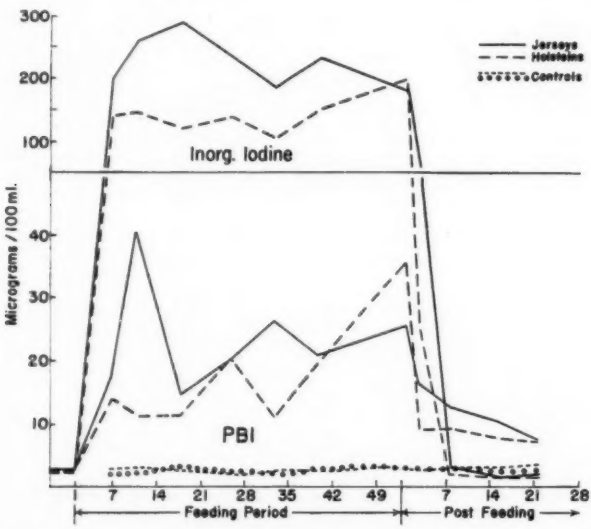

FIG. 3. The effect of feeding thyroprotein on the changes in serum inorganic iodine and protein-bound iodine in Jersey vs. Holstein cows. Data from 15 and 25 g. / 1,000 lb. / day dosages combined. See Experiment II.

the Jersey and Holstein which received 25 g. per 1,000 lb. body weight maintained both a higher inorganic-iodine level and a higher PBI level than did the Jersey and Holstein receiving 15 g. of thyroprotein per 1,000 lb. body weight. The inorganic iodine fraction, however, returned to normal within a week after the termination of thyroprotein feeding, but the PBI levels decreased more gradually. Although not shown, data for the ensuing 12 weeks showed a gradual return to normal during this period.

In Figure 3 the hereditary effect due to the difference between breeds on the level of inorganic iodine and PBI is shown. The combined values of the Jerseys receiving respectively 15 and 25 g. per day per 1,000 lb. body weight are compared to the values of the Holsteins fed the same dosages. At the beginning of the trial the inorganic iodine of the two Jerseys was elevated more by thyroprotein feeding on the body weight basis than was that of Holsteins. There was an obliteration of this effect by the end of the trial, the reason for which is not apparent. There would seem to be no marked hereditary differ-

ence due to breed in PBI response when it is considered that a high value for one Jersey (63 γ %) resulted in the high average shown 10 days after thyroprotein feeding began. Based on the results of Reece and Mann (15), the small differences due to breed might have been eliminated by use of the n-butanol extraction method.

Experiment III. Effect of thyrotropic hormone on PBI and inorganic iodine. In order to study by more natural means the effect of thyroid stimulation on blood inorganic and protein-bound iodine, 25 mg. of thyrotropic hormone [3] was injected subcutaneously into an Ayrshire steer on two successive days. The results are shown in Table 2.

TABLE 2

The effect of thyrotropic hormone injection on blood inorganic iodine and protein-bound iodine

Time after injection	Inorganic iodine	PBI
(Days)	*(γ%)*	*(γ%)*
1[a]	1.0	3.2
2[a]	1.0	5.2
3	1.0	6.1
5	1.0	4.8

[a] 25 mg. thyrotropic hormone injected immediately after bleeding.

In this experiment the PBI was raised from a normal level to a mildly hyperthyroid level by thyrotropic stimulation of the animal's own thyroid gland. In spite of the rise in the PBI it is of interest to note that the inorganic fraction remained at 1.0 γ %, indicating that it is independent of the PBI level under these conditions. This more nearly represents the relationship between inorganic iodine and PBI found in clinical hyperthyroidism, where a PBI of 10 to 20 γ % usually occurs along with a normal inorganic iodine level, which for humans is 1.0-3.0 γ % (14) when determined by this method.

Although the PBI test was not an accurate indicator of the level of thyroid activity during and after thyroprotein feeding, the changes that did occur were sufficiently drastic to leave no doubt that thyroprotein feeding had raised the PBI level. After thyroprotein feeding was terminated, PBI remained at a rather high level, compared to the normal range, for several months. Thus it should be possible for an extended period following the termination of feeding to detect cows that had been fed this substance.

The feeding of thyroprotein to cows on official-production test is prohibited by the various breed registry associations because it is considered to be an artificial stimulant to milk production. The PBI test offers a possible practical solution to the need for a method of identifying cows that are being fed thyroprotein. The inorganic-iodine test, although less specific, could be used to detect cows being fed thyroprotein, or some other material high in iodine, but would be of no value a few days after the feeding was terminated.

[3] Supplied by Armour and Co., Chicago (lot No. K-39405-R, a lyophylyzed preparation).

SUMMARY AND CONCLUSIONS

The development of materials having the ability either to increase or decrease thyroid activity in animals has focused attention on the need for a simple laboratory test which can be used as an accurate indicator of thyroid activity in the regulation of thyroid status. Accordingly, the changes in blood serum protein-bound iodine (PBI), found under normal conditions to reflect the amounts of thyroid hormone in the blood, and plasma cholesterol, shown to have an inverse relationship to thyroid activity, were measured during and after the feeding of different dosages of thyroprotein (Protamone) to dairy cows. Inorganic iodine and blood sugar determinations also were made.

During thyroprotein feeding both inorganic iodine and PBI values increased tremendously above the normal range. The increase in PBI, which was much higher than would be expected from any clinical indications of thyroid activity observed, is partly attributed to the formation of noncalorigenic protein-bound iodine resulting from the combination of inorganic iodine in the thyroprotein with certain blood protein constituents and also the presence of iodinated compounds other than thyroxine in the thyroprotein. This explanation is supported by the small increase in PBI without an increase in the inorganic-iodine level when the thyroid is stimulated by thyrotropic hormone.

After thyroprotein feeding was terminated, inorganic iodine decreased rapidly to normal, whereas PBI persisted for several months at a level higher then the prefeeding values.

Plasma cholesterol decreased for a time during thyroprotein feeding and then gradually returned to normal. After thyroprotein feeding was terminated, cholesterol rose above the normal level and then gradually returned to normal. Although plasma cholesterol appeared to reflect more accurately the thyroid status of the cows than did PBI, particularly after thyroprotein feeding was terminated, neither of these blood constituents could be accurately correlated with dosage of thyroprotein. The possible reasons for these changes are discussed.

No changes in blood sugar due to thyroprotein feeding were observed. The injection of thyrotropic hormone into a steer resulted in an increase in PBI without any increase in inorganic iodine. This is further indication that PBI does reflect thyroid status when thyroid activity is increased without the interference of noncalorigenic PBI.

REFERENCES

(1) Blaxter, K. L., Reineke, E. P., Crampton, E. W., and Petersen, W. E. The Role of Thyroidal Materials and of Synthetic Goitrogens in Animal Production and an Appraisal of their Practical Use. J. Animal Sci., **8**: 307-352. 1949.

(2) Connor, A. C., Swenson, R. E., Park, C. W., Gangloff, E. C., Lieberman, R., and Curtis, G. M. The Determination of the Blood Iodine. Surgery, **25**: 510-517. 1949.

(3) Curtis, G. M., and Fertman, M. B. Iodine in Nutrition. J. Am. Med. Assoc., **139**: 17. 1949.

(4) EPSTEIN, A. A., AND LANDE, H. Studies on Blood Lipoids. I. The Relation of Cholesterol and Protein Deficiency to Basal Metabolism. Arch. Internal Med., **30**: 563-577. 1922.

(5) FOLDES, F. F., AND MURPHY, ANNA J. Distribution of Cholesterol, Cholesterol Esters and Phospholipid Phosphorous in Blood in Thyroid Disease. Proc. Soc. Exptl. Biol. Med., **62**: 218-223. 1946.

(6) FRIEDBERG, W., AND REINEKE, E. P. Thyroxine-like Compounds of Iodinated Protein and Thyroid Gland. Fed. Proc., **11**: 50. 1952.

(7) GILMORE, L. O., LONG, J. F., HIBBS, J. W., AND ELY, F. Thyroid Status. Ohio Farm and Home Research, **37**: 92, 99. 1952.

(8) HIBBS, J. W., AND KRAUSS, W. E. Thyroprotein Feeding Affects Blood Cholesterol. Ohio Agr. Expt. Sta. 66th Ann. Rpt., Bull. **674**: 16-17. 1948.

(9) KUNKLE, H. L., COLBY, R. W., AND LYMAN, C. M. The Relationship of Serum Protein-Bound Iodine Levels to Rates of Gain in Beef Cattle. J. Animal Sci., **12**: 3-9. 1953.

(10) LEWIS, R. C., AND RALSTON, N. P. Protein-Bound Iodine Levels in Dairy Cattle Plasma. J. Dairy Sci., **36**: 33-38. 1953.

(11) LONG, J. F., GILMORE, L. O., CURTIS, G. M., AND RIFE, D. C. The Bovine Protein-Bound Iodine and its Relation to Age and Breed. J. Dairy Sci., **35**: 603-606. 1952.

(12) MEANS, J. H. *The Thyroid and its Diseases.* 2nd ed., p. 165. J. B. Lippincott Co., Philadelphia, Pa. 1948.

(13) MULLER, G. L. The Clinical Significance of Cholesterol. Am. J. Digest. Diseases, **12**: 355-377. 1945.

(14) RAPPORT, R. L., AND CURTIS, G. M. The Clinical Significance of the Blood Iodine: A Review. J. Clin. Endocrinol., **10**: 735-790. 1950.

(15) REECE, R. P., AND MANN, E. B. Serum Precipitable and Butanol Extractable Iodine of Bovine Sera. Proc. Soc. Exptl. Biol. Med., Proc., **79**: 208-210. 1952.

(16) REINEKE, E. P., AND TURNER, C. W. Formation *in vitro* of Highly Active Thyroproteins, their Biologic Assay and Practical Use. Mo. Agr. Expt. Sta. Research Bull. 355. 1942.

(17) REINEKE, E. P., WALLACH, D. P., AND WOLTERINK, L. F., Thyroxine Content of Synthetic Thyroprotein as Determined by a Radioactive Isotope Dilution Technic. J. Dairy Sci., **33**: 386-387. 1950.

(18) SAIFER, A., AND KAMMERER, O. F. Photometric Determination of Total Cholesterol in Plasma or Serum by a Modified Liebermann-Burchard Reaction. J. Biol. Chem., **164**: 657-677. 1946.

THE CORRELATION OF MAMMARY GLAND DEVELOPMENT OF HEIFERS WITH THEIR BODY WEIGHT AND THE MILK PRODUCTION OF THEIR DAMS [1, 2]

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Studies conducted by Swett *et al.* (7, 8) have indicated that the mammary tissue development of young heifer dairy calves may serve as a criterion for the selection of those animals which may later be the most productive. Schabinger *et al.* (5) found a statistically highly significant relationship between the extent of mammary tissue development and body weight of Holstein and Guernsey heifers at 3 months of age. As reported by Brown (1), the relationship between mammary tissue size and body weight appears to be more significant at 4 months of age for both width and length of the mammary tissue studied than at any other age from 3 to 6 months.

EXPERIMENTAL PROCEDURE

The studies presented in this report were conducted in two parts in an effort to determine the relationship between the mammary gland tissue development of young heifer calves and their dams' milk production as well as with their own body weight. The production records on the dams were those made in the Dairy Herd Improvement Association testing program. All records were adjusted to a 305-day, twice-a-day milking, mature equivalent basis and then converted to fat-corrected milk.

Part I. Data were collected on 96 heifer calves born in the Pennsylvania State College herd from October 1949 to July 1951. They consisted of 22 Ayrshire, 11 Brown Swiss, 21 Guernsey, 31 Holstein, and 11 Jersey heifers. The mammary glands were measured by palpation when the calves were 4 months of age. This age was used in accordance with previous reports (7, 8). The udders were classified as being in the quarter or half stage. Udders were considered in the quarter stage when each quarter could be palpated separately and in the half stage when the mammary tissue of the front and rear glands was attached firmly together. The length and width measurements were taken for each quarter when in the quarter stage.

Part II. The heifers in this part of the study were from the Pennsylvania State College Herd and seven state institutions located throughout Pennsylvania.

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[1] Data contained in this publication are from a thesis submitted by the senior author to the Graduate School of the Pennsylvania State College in partial fulfillment of the requirements for the degree of Master of Science.

[2] Authorized for publication as paper No. 1787 in the Journal series of the Pennsylvania Agricultural Experiment Station.

All heifers were purebred Holsteins with a total of 34 calves in the 2 months of age group and 48 calves in the 3, 4, and 5 months of age groups. The 34 calves measured at 2 months of age were carried through the 3, 4, and 5 months periods with 14 calves added when they were 3 months of age. All heifers were measured at monthly intervals to study the amount and rapidity of growth of the mammary tissues in relation to body weight.

EXPERIMENTAL RESULTS

Part I. This study indicates that differences may exist between the five major dairy breeds as to the rate of mammary gland development and as to the per cent of calves in the quarter or half stages when the mammary glands are palpated at 4 months of age. The per cent of calves in the half stage appeared to be related directly to the earliness of maturity for that particular breed, as seen in Table 1, with the earlier maturing breeds having a higher percentage of calves in the advanced stage at this age.

The data indicate that the udders in the quarter stage had a greater combined length than those in the half stage. It appears that the length of the two quarters cannot be added together to obtain a combined length comparable to that of the

TABLE 1

Summary of udder measurements (Part I), stage of udder development and the dam's average milk production for heifer calves of the 5 major dairy breeds measured at 4 months of age

Breed	Stage	No. in stage	% in stage	Av. length	Av. width	Total records used	Av. cwt. FCM
				(cm.)	*(cm.)*		
Ayrshire	Quarter	13	59.1	6.4	2.6	19	111.2[a]
	Half	9	40.9	5.9	3.4	20	117.4
Brown Swiss	Quarter	8	72.7	5.9	2.5	15	116.1
	Half	3	27.3	4.9	3.1	5	123.0
Guernsey	Quarter	8	38.1	7.9	3.0	23	108.3[b]
	Half	13	61.9	6.7	3.6	29	109.2
Holstein	Quarter	16	51.6	6.1	2.3	35	137.5
	Half	15	48.4	6.4	3.3	33	149.4[c]
Jersey	Quarter	1	9.1	7.2	2.4	3	132.0
	Half	10	90.0	6.0	3.4	20	101.8[d]

[a] Records from 12 cows
[b] Records from 7 cows
[c] Records from 13 cows
[d] Records from 9 cows

half stage because of the position in which the glands lie. Also, the data in Table 1 indicate that for each breed the heifers in the half stage were from dams which had a higher production than the heifers in the quarter stage, excluding the Jersey breed, in which only one heifer was in the quarter stage at that age.

Correlation coefficients were calculated between the average width of gland

TABLE 2

Summary of correlation coefficients (r) and linear regressions (b) of mammary gland development of heifer calves at 4 months of age and their dams' milk production (Part I)

Breed	Stage	Degrees freedom	Mean width of udder and dam's milk production		Mean length of udder and dam's milk production	
			(r)	*(b)*	*(r)*	*(b)*
Ayrshire	Quarter	10	0.6303[a]	0.0200	0.0613	0.0062
	Half	7	0.1012	0.0029	0.3330	0.0010
	Combined	19	0.3241	0.1301	0.1032	0.0062
Brown Swiss	Quarter	6	0.1609	0.0015	0.0015	0.0006
	Half	1	—0.5393	—0.0167	—0.4573	—0.0274
	Combined	9	—0.1306	—0.0029	—0.2115	—0.0108
Guernsey	Quarter	5	0.4704	0.0325	0.6819	0.0898
	Half	11	—0.5330	—0.0269	—0.3524	—0.0295
	Combined	18	—0.1770	—0.0109	—0.0104	—0.0011
Holstein	Quarter	14	—0.3400	—0.0110	—0.4447	—0.0341
	Half	11	—0.0023	—0.0005	0.4120	0.0136
	Combined	27	0.0280	0.0010	—0.1379	—0.0081
Jersey	Quarter	0	.....	.....	.....	.....
	Half	7	0.5641	0.0333	0.1912	0.0105
	Combined	8	0.1974	0.0102	0.4149	0.0210

[a] Significant at the 5% level

as well as the average length of gland with the dam's average production in hundredweight of fat-corrected milk, as presented in Table 2. These were computed for the quarter stage, half stage, and the combined stages for each breed. The correlation coefficients, as presented in Table 2, indicate that no relationship existed between any of the mammary gland measurements at 4 months of age and the dam's average milk production for any of the breeds observed in these studies.

Part II. The heifers in this part of the study were measured at 2, 3, 4, and 5 months of age; the data are presented in Tables 3, 4, and 5. At 2 and 5 months of age, it was found that the length of the glands of the udders in the half

TABLE 3

Summary of udder measurements of Holstein heifers at 2, 3, 4, and 5 months of age, stage of growth, average body weight and the dam's average milk production (Part II)

Age group	Stage	No. in stage	% in stage	Av. length	Av. width	Total records used	Av. ewt. FCM	Body weight of heifers	
(mo.)				*(cm.)*	*(cm.)*			*(lb.)*	*(% normal)*[a]
2	Quarter	32	95.4	2.8	1.3	86	113.6	185.0	123.3
	Half	2	4.6	3.6	2.0	3	92.0	212.0	141.3
3	Quarter	31	65.5	4.6	1.9	79	113.1	224.4	112.2
	Half	17	34.5	4.5	2.6	39	113.5	264.4	132.2
4	Quarter	11	24.1	6.3	2.5	35	116.5	276.1	110.4
	Half	37	75.9	5.8	3.3	83	112.9	309.7	123.9
5	Quarter	1	3.4	5.0	2.2	2	104.0	319.0	103.7
	Half	47	96.6	6.6	3.7	116	113.9	351.4	114.3

[a] Calculated on basis of Morrison standards (3)

TABLE 4

Summary of correlation coefficients (r) and linear regressions (b) of mammary gland development of Holstein heifers at 2, 3, 4, and 5 months of age and their dam's milk production (Part II)

Age group	Stage	Degrees freedom	Mean width of udder and dam's milk production		Mean length of udder and dam's milk production	
(mo.)			*(r)*	*(b)*	*(r)*	*(b)*
2	Quarter	30	—0.0021	—0.0004	0.0011	0.0004
	Half	0	.....	.....	.....	.....
	Combined	30	—0.0990	—0.0018	0.0719	0.0027
3	Quarter	29	—0.0253	—0.0005	—0.0064	—0.0003
	Half	15	—0.0135	—0.0002	0.0855	0.0022
	Combined	46	—0.0202	—0.0005	—0.0134	—0.0006
4	Quarter	9	0.0806	0.0039	—0.1289	—0.0162
	Half	35	—0.1294	—0.0024	0.1762	0.0061
	Combined	46	—0.1143	—0.0030	0.1109	0.0053
5	Quarter	0	.....	.....	.....	.....
	Half	45	—0.0817	—0.0015	0.0759	0.0025
	Combined	45	—0.0429	—0.0009	0.0930	0.0033

TABLE 5

Summary of correlation coefficients (r) and linear regressions (b) of mammary gland development of Holstein heifers at 2, 3, 4, and 5 months of age and their body weight (Part II)

Age group	Stage	Degrees freedom	Mean width of udder and body weight		Mean length of udder and body weight	
(mo.)			*(r)*	*(b)*	*(r)*	*(b)*
2	Quarter	30	0.3714[a]	0.0055	0.5083[b]	0.0173
	Half	0	.....	.....	.....	.....
	Combined	30	0.4172[a]	0.0067	0.5171[b]	0.0178
3	Quarter	29	0.2731	0.0040	0.3532[a]	0.0138
	Half	15	0.3680	0.0045	0.1609	0.0029
	Combined	46	0.4905[b]	0.0075	0.2223	0.0035
4	Quarter	9	0.8272[b]	0.0147	0.7326[b]	0.0340
	Half	35	0.5273[b]	0.0060	0.3902[a]	0.0083
	Combined	46	0.6476[b]	0.0093	0.3538[a]	0.0093
5	Quarter	0	.....	.....	.....	.....
	Half	45	0.5085[b]	0.0053	0.3740[b]	0.0070
	Combined	45	0.5866[b]	0.0067	0.4088[b]	0.0080

[a] Significant at the 5% level
[b] Significant at the 1% level

stage exceeded those in the quarter stage, but during the other 2 months the reverse was true and further substantiated results obtained in Part I of the study.

Milk production of the dams of the heifers was correlated with mammary development to determine whether such a direct relationship existed. From the correlation coefficients presented in Table 4, it appears that there was no direct relationship between the mammary gland development of the calf at 2, 3, 4, or 5 months of age and the dam's milk production.

The heifers in this part of the work also were taped for body weight at

monthly intervals beginning at either 2 or 3 months of age, depending upon the age when the animal was first included in the study. When the mean width of the glands was correlated with the body weights, a significant correlation coefficient was obtained when the heifers were 2 months of age and highly significant correlation coefficients at the 4 and 5 month levels (Table 5). In the 4 and 5 months of age groups the correlation coefficients indicate that a significant part of the variance of udder size can be explained by differences in body weight. Correlation coefficients at 2, 4, and 5 months of age indicate that the development of the length and width of the glands is in direct relationship to the body weight. When the calves were 3 months of age, there was no significant correlation between the measurements of the mammary tissues in the quarter and half stages and body weight. Only when these measurements were combined was there a highly significant correlation at this age. Explanation of this relationship does not appear to be available except on the basis of increased number of observations when all calves were combined at this age.

SUMMARY

A study has been conducted to determine if a relationship exists between the mammary gland development of young heifer calves from 2 to 5 months of age with their body weight and their dam's milk production. A direct relationship appeared to be present between the per cent of calves in the half stage of mammary gland development at 4 months of age and the earliness of maturity for that particular breed.

There was no direct relationship between the mammary gland measurements of heifers at 2, 3, 4, or 5 months of age and the milk production of the dams. Statistical analysis of the relationship between the mammary gland measurements and the body weight of heifers indicate that a highly significant relationship existed at 4 and 5 months of age. This indicates that under the conditions of this study the size of the mammary tissue is related in part to the size of the animal at these ages.

REFERENCES

(1) Brown, D. C. Udder Development in Young Heifer Calves. J. Dairy Sci., 34: 367-373. 1951.

(2) Davis, H. P., Morgan, R. F., Brody, S., and Ragsdale, A. C. Relationship of Height at Withers and Chest Girth to Live Weight of Dairy Cattle of Different Breeds and Ages. Nebr. Agr. Expt. Sta. Bull. 91. 1937.

(3) Morrison, F. B. *Feeds and Feeding.* 21st ed., p. 766. The Morrison Publishing Co., Ithaca, N. Y. 1948.

(4) Ragsdale, A. C. Estimating Live Weights of Dairy Cattle. Mo. Agr. Expt. Sta. Bull. 354. 1935.

(5) Schabinger, J. R., and Knodt, C. B. The Relationship of Mammary Gland Development and Body Weight. J. Dairy Sci., 31: 95-98. 1948.

(6) Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(7) Swett, W. W. A Cow a Calf Will Be. Yearbook of Agriculture, 1943-47. pp. 197-200.

(8) Swett, W. W., and Matthews, C. A. Dairy Cow's Udder Studied to Establish Developmental Standards. Yearbook of Agriculture, 1934. pp. 175-181.

HERITABILITY OF FERTILITY IN DAIRY CATTLE [1]

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Low reproductive efficiency of dairy cattle has long been recognized as a cause of serious economic loss to the dairy farmer, and it has seemed reasonable to assume that improvement in reproductive efficiency could be attained by selecting for some measure of this trait. If dairy cattle breeders are to select for high fertility measured in some particular manner—in addition to selecting for other traits of economic importance—it is important to know how effective selection for that particular measure of fertility can be, what the best selection procedure is, and what relative emphasis should be placed on fertility and other traits of economic importance. In order to answer such questions, it is necessary to have reliable estimates of the repeatability and heritability of the particular measure of reproductive efficiency which is to be used.

In recent years a considerable amount of work has been done on this problem, and the measures of fertility most frequently employed have been the number of services required for conception, nonreturns to first service—which is undoubtedly highly correlated with the former—and in some instances, calving interval. Most of the earlier work has been done within certain experiment station herds, but in the last few years several investigations have dealt with populations of dairy cattle being bred artificially by bulls belonging to artificial breeding cooperatives.

One of the earlier reports of the work on the problem of infertility is that of Spielman and Jones (5) in which they reported a correlation, $r = +0.546 \pm 0.118$, between the reproductive efficiency of the foundation cows in the Oregon State College herd and the mean reproductive efficiency of their female descendants. It is not clear, however, whether or not this correlation is biased upward by the breed differences which they report.

Although Tanabe and Casida (6) and Trimberger and Davis (7) do not present estimates of repeatability of the number of services required per conception, it is clear from their data that repeatability is very low. This is inferred from their finding that the number of services required for previous conceptions is of no practical value in predicting the number of services required for later conceptions and of still less value for predicting the services required for conception by their progeny.

Olds *et al.* (3) have reported similar results from a study of the Kentucky Agricultural Experiment Station herd, and recently Olds and Seath (4) have

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reported a correlation, $r = 0.084 \pm 0.012$, between the number of services required per conception by dairy cattle bred artificially by the Kentucky Artificial Breeding Association in two consecutive years. Leonard (2) studied the services required per conception in a population of dairy cattle bred artificially in Maine, and from these data he estimated repeatability to be 0.075.

SOURCE AND ANALYSIS OF DATA

Nonreturns to first service. The data utilized in this study were obtained by checking a list of cows sired artificially by Holstein bulls in New York against the records of the New York Artificial Breeders' Cooperative, Inc., to determine which of these cows had been inseminated artificially and what the outcome of the first service had been. Because the list of cows was obtained from the Dairy Records' Office, this study included production tested cows only. As many first services were observed for each animal as could be found in the records of the New York Artificial Breeders' Cooperative, Inc. If a cow had not returned to first service within 180 days, the observation was assigned the value *1*, and if the cow had returned, that observation was assigned the value *0*. The particular value assigned to a return and a nonreturn has no bearing on the estimates of repeatability and heritability, and the numbers *1* and *0* were chosen as a matter of convenience.

To facilitate the analysis of these data, they were punched on I.B.M. cards, each card containing the following information:

1. Identification of the cow.
2. Identification of the sire of the cow.
3. Identification of the herd in which the cow was located.
4. The year-season [3] in which the insemination was made.
5. The coded value of a single first service of the cow, *0* or *1*.

A total of 1,833 observations was made on 1,015 cows located in 297 different herds throughout the state. These 1,015 cows were sired by 52 different Holstein bulls. The inseminations observed were made in a total of 33 different year-seasons.

The analysis of these data consisted of estimating certain components of variance by an estimation procedure designated as Method 1 in a paper by Henderson (1). This estimation procedure results in unbiased estimates of variance components from nonorthogonal data under the assumption of uncorrelated random variables.

The components of variance estimated from these data on nonreturns to first service were σ^2_s, σ^2_h, σ^2_y, σ^2_c, σ^2_{sh}, σ^2_{hy}, and σ^2_e.

σ^2_s is the variance due to sires. The difference among sires which would contribute to the variation among nonreturns of their daughters are additive

[3] The year-season term includes differences associated with years and differences among seasons within years. The months of the year were grouped into four seasons with an attempt to group them such that seasonal variations would be maximized. The four seasons were Dec., Jan., Feb.; Mar., Apr., May; June, July, Aug.; and Sept., Oct., Nov.

genetic differences plus a certain fraction of epistatic differences. This results from the fact that each of the cows observed had received a sample one-half of the genotype of their respective sires. Because each sire had daughters located in many different herds and the herds contained daughters of one or more sires, it was possible to separate variation due to genetic differences among sires from variation due to genetic and environmental differences among herds.

σ^2_h is the variance due to herds. Differences which may exist among herds include genetic differences, differences due to genetic and environmental interaction and differences in herd environments. Such things as the ability of herd owners to detect estrus and the skill of inseminating technicians, as well as other management factors, are possible sources of environmental differences among herds which may contribute to variation in nonreturns to first service.

σ^2_y is the variance due to year-seasons, and results from changes in the average environment of the population from one year-season to another. Environmental changes from season to season within years and environmental changes associated with different years are included in this component. Any effects of seasonal changes in light, temperature, and weather would be included, as well as any effects due to changes in the techniques of semen processing and artificial insemination.

σ^2_c is the variance among paternal half-sisters located in the same herd. It is due to genetic differences which include deviations due to dominance and epistasis in addition to additive genetic differences, and what may be called permanent environmental effects. Permanent environmental differences result from those modifications of the cows' inherent fertilities brought about by the environments to which they have been subjected during their lifetimes. Such things as permanent injuries due to accident or disease come to mind most readily.

σ^2_{sh} is the variance due to sire-herd interaction. Any interactions which may have occurred between the genotypes of the sires studied and the environment of the herds in which their daughters were located and/or the genetic constitution of these herds, as represented by the mates of the sires, would be included in this component of variance.

σ^2_{hy} is the variance due to interaction between herds and year-seasons. Any interactions between the genetic constitutions of individual herds and seasonal environmental effects would be included in this component of variance. The effect of changes in the environment of individual herds due to changes in management practices would also be included in this component.

σ^2_e is the variance due to all remaining, nonidentified sources of variation. The quality of the semen used for insemination and temporary infection of the individual at the time of service are two such nonidentified sources of variation. There are perhaps many such factors which are not known.

Calving interval. After completing the analysis of the data in which nonreturns to first service were utilized as the measure of fertility of cows sired artificially by Holstein bulls, another sample was studied with calving interval

as the measure of fertility. This second group of Holstein cows differed somewhat from those in which nonreturns to first service were used as the measure of fertility because it was not necessary for the second group to have been inseminated artificially. However, it was necessary for each cow to have freshened at least twice.

From these data it was possible to make up a calving interval card for each cow having two or more production records by subtracting the first freshening date from the second. Only the first two freshening dates were considered for any cow or, in other words, there was but a single observation on each cow. Each of the cards made up for the study had the following information punched on it.

1. The identification of the cow.
2. The identification of the sire of the cow.
3. The identification of the herd in which the cow was located.
4. The length of the calving interval measured in months.

These data included 1,036 cows sired artificially by 57 different bulls on 281 farms.

The analysis of the data on calving interval was exactly the same as the analysis of the data on nonreturns to first service with the exception that fewer components of variance were estimated. As may be seen from an examination of Table 1, the data on nonreturns to first service indicated that

TABLE 1
Components of variance and percent of total variance (Nonreturns)

Components	Estimated value	Percent of total
σ^2_s	0.0002	0.1
σ^2_h	—0.0015	0.0
σ^2_y	0.0002	0.1
σ^2_e	0.0063	2.6
σ^2_{sh}	0.0056	2.3
σ^2_{hy}	0.0092	3.7
σ^2_*	0.2255	91.3

certain sources of variation were probably of no consequence. Also, because there was but a single calving interval observed for each individual, it was impossible to separate variation due to genetic and permanent environmental differences from other sources of variation among paternal half-sisters within a herd. Consequently, the total variation among paternal half-sisters in the same herd is called σ^2_e in the calving interval study. Other components of variance which were estimated from the data on calving interval were σ^2_s, σ^2_h, and σ^2_{sh}. The interpretation of these components of variance is essentially

the same as described for the components estimated from the data on nonreturns to first service.

RESULTS AND DISCUSSION

In the data on nonreturns to first service, the number of daughters per sire ranged from 1 to 212 with an average of 35.2. The mean nonreturns per sire ranged from 0 to 1, and the mean of all observations was 0.567 or 56.7% nonreturns to first service.

The estimates of the components of variance from these data and the per cent of the total variance which each of them contributes are presented in Table 1. Having obtained estimates of the different components of variance, it was possible to use certain of them to estimate the repeatability and heritability of fertility as measured by nonreturns to first service.

Since σ^2_c in Table 1 is an estimate of the variance among paternal half-sisters within the same herd, an estimate of the repeatability of nonreturns to first service in such a population of half-sisters may be computed from the ratio $\sigma^2_c/[\sigma^2_c + \sigma^2_e] = 0.0063 \;/\; [0.0063 + 0.2255] = 0.027$. An estimate of repeatability in a population of cows in the same herd, but each having a different sire, may be computed from the ratio $[\sigma^2_c + \sigma^2_s + \sigma^2_{sh}] \;/\; [\sigma^2_c + \sigma^2_s + \sigma^2_{sh} + \sigma^2_e]$. Repeatability of nonreturns to first service for such a population as this is estimated to be 0.051. Since most herds are composed of paternal half-sisters, a few more closely related pairs, and many more less closely related pairs, the intra-herd repeatability of nonreturns to first service would be estimated to be some value between 0.027 and 0.051.

An estimate of the heritability may be computed also from the estimates of the components of variance in Table 1. The ratio used to estimate heritability is $\sigma^2_g/[\sigma^2_g + \sigma^2_e]$. Although σ^2_g is not estimated directly from the data, it may be estimated indirectly from the relationship $\sigma^2_s \cong \frac{1}{4}\ \sigma^2_g$. This relationship is an approximation rather than an equality because σ^2_s contains some variation due to epistasis in addition to additive genetic differences while σ^2_g represents only additive effects. This computation yields a heritability estimate of 0.004. This is an estimate of the intraherd heritability of nonreturns to first service in a population with intraherd additive genetic variance equal to the additive genetic variance among sires used in artificial breeding but with no nonadditive genetic variance, and no variance due to permanent environmental effects. Consequently, 0.004 is an overestimate of the heritability of nonreturns to first service.

Turning to the analysis of the data on calving intervals, it was found that the number of daughters per sire ranged from 1 to 110 with a mean of 18.2 The mean calving interval per sire ranged from 11.00 to 17.25 with a population mean of 13.08 months.

The estimates of the components of variance from the calving interval data and the per cent of the total variance which each component represents are presented in Table 2. The estimate of σ^2_s in Table 2 is —0.10, but because a

TABLE 2
Components of variance and percent of total variance (Calving interval)

Component	Estimated value	Percent of total
σ^2_s	—0.10	0.0
σ^2_h	—0.33	0.0
σ^2_{hs}	1.97	21.8
σ^2_e	7.05	78.2

negative variance is impossible by definition, the most plausible interpretation of such an estimate is to attribute the negative sign to sampling error and consider it to be an estimate of zero variance. When the estimate of σ^2_s is interpreted in this way it immediately follows that the heritability of calving interval is estimated from these data to be zero.

The most striking feature of Tables 1 and 2 is the large percentage of the total variation attributable to error. This reveals that in these analyses the factors which can be identified and measured are apparently of little consequence as sources of variation among nonreturns to first service and calving intervals, whereas nonidentifiable and nonmeasurable factors grouped together as sources of error are the cause of a very large part of the total variation in these measures of fertility.

There are certain characteristics of the data on nonreturns and calving interval which should be noted. First of all, the data were limited to production tested cows. Perhaps the most significant consequence of this is that heifers which never conceived were not included in the sample. How much bias this introduces into the above estimates of heritability depends upon the extent to which this phenomenon is dependent upon genetic differences among sires. Perhaps estimates of heritability obtained from data which do not include heifers which never conceived are more desirable than estimates from data which do include such females since the measures of fertility usually employed are not applicable to the latter animals, and any selection practiced would be based on these measures. A further limitation of these data is that nonreturns to first service and calving interval, the measures of fertility used in this study, fail to evaluate the significance of the viability or nonviability of the calf resulting from service. This, however, is a matter of choosing the appropriate measure of reproductive efficiency.

Another pertinent consideration concerns the limitations of the method of analysis. If the assumptions concerning the distribution of the variables are valid, then it may be stated with certainty that the method results in unbiased estimates of the components of variance. To assume that the effects of consecutive periods of time are independent and that seasonal effects are random variables is probably not entirely valid. It is possible, however, that removal

of the bias in the estimates resulting from this invalid assumption would not justify the increased complexity of the analysis which would be required if year-seasons had been considered as fixed effects.[4] A further unknown in the method of analysis utilized in this study concerns the sampling errors of the estimates of components of variance. Therefore, it is impossible to compute confidence limits. When the sample size is large, as in this instance, the sampling errors are probably quite small.

In addition to the limitations imposed by the nature of the data and the manner in which they were collected and analyzed, there is the question as to whether or not the genetic variance with respect to fertility is less in bulls selected for use in artificial breeding than in the population as a whole. But, there are reasons for thinking that the genetic variance for fertility among the bulls studied was not reduced appreciably by selection. When bulls are selected for use in artificial breeding, considerably more emphasis is placed on transmitting ability for production than on transmitting ability for fertility. Of course, the fertility of a bull's semen is given considerable emphasis. Although there is frequently considerable information with which to appraise the fertility of the bull's semen, there is usually very little information which might be used to appraise the bull's transmitting ability for fertility. These considerations, plus the evidence from other studies that repeatability and heritability for the more common measures of fertility are quite low, indicate that selection for inherent fertility of the bulls brought into artificial breeding is relatively ineffective. Consequently, it seems valid to assume that the genetic variance among the sires studied with respect to fertility is essentially the same as that which exists among individuals which might be included in a random sample of Holstein bulls.

It seems certain from this study, and from those reviewed briefly, that genetic variance with respect to fertility is essentially zero when fertility is measured by nonreturns to first service, services required per conception, or by length of calving interval. This does not preclude the possibility that there exists appreciable genetic variance of some other measures of reproductive efficiency. If some measure of fertility such as the number of viable calves produced per month of reproductive life were employed, the estimate of genetic variance might be considerably different from that for nonreturns and calving interval. Certainly such a measure of fertility would evaluate the presence or absence of lethal and sublethal genes which result in nonviable calves. Whether or not these different deleterious genes present a serious problem depends considerably on what the frequencies of such genes are in the population. By the very nature of their action, it would be expected that their frequencies would be very low, but it would be informative to have estimates of genetic variance of some measure which reflected the presence or absence of these kinds of genes.

[4] The reader is referred to Henderson (1), Methods 2 and 3 for indications of the increased complexity of the analysis necessary if certain elements are regarded as fixed.

SUMMARY AND CONCLUSIONS

Utilizing data obtained from the Dairy Records' Office in the Animal Husbandry Department at Cornell University and the New York Artificial Breeders' Cooperative, Inc., it has been possible to study the relative reproductive efficiency of groups of artificially sired paternal half-sibs located on different farms throughout New York state. Because these sires had daughters on many different farms, it was possible to separate variation due to differences among herds from variation due to differences among sires. The analytical procedure consisted of estimating the variance due to sires, herds, year-seasons, cows, and certain interactions.

Based on the estimates of the components of variance, repeatability of fertility measured by nonreturns to first service in a population of half-sisters within the same herd is estimated to be 0.027, and repeatability of this measure in a population of cows by different sires within the same herd is estimated to be 0.051. The average intraherd repeatability is estimated to be some value between 0.027 and 0.051. The heritability of nonreturns to first service is estimated to be 0.004. When calving interval is used as the measure of fertility, heritability is estimated to be zero.

On the basis of this work and the work of others which has been reviewed briefly, it seems certain that selection for fertility measured by nonreturns to first service, services required per conception, or calving interval cannot be very effective and that consideration given to fertility in selecting breeding stock will but serve to decrease the effectiveness of selection for such traits as butterfat production and type conformation. A further implication of these results is that any marked improvement obtained in the reproductive efficiency of the dairy cattle population must be brought about by improvement of nutritional, pathological, and/or other environmental factors which exert an influence on the process of reproduction.

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REFERENCES

(1) Henderson, C. R. Estimation of Variance and Covariance Components. Biometrics. In Press.

(2) Leonard, H. A. An Analysis of Factors Affecting Artificial Breeding Efficiency of Dairy Cattle in Maine. Unpublished Research Problem. 1950.

(3) Olds, D., Morrison, H. B., and Seath, D. M. Efficiency of Natural Breeding in Dairy Cattle. Kentucky Agr. Expt. Sta. Bull. 539. 1949.

(4) Olds, D., and Seath, D. M. Predicting the Breeding Efficiency of Dairy Cattle. J. Dairy Sci., 32: 376. 1950. (Abs.)

(5) SPIELMAN, A. A., AND JONES, I. R. The Reproductive Efficiency of Dairy Cattle. J. Dairy Sci., 22: 329-334. 1939.

(6) TANABE, R., AND CASIDA, L. E. The Nature of Reproductive Failures in Cows of Low Fertility. J. Dairy Sci., 32: 237-246. 1949.

(7) TRIMBERGER, G. W., AND DAVIS, H. P. Predictability of Breeding Efficiency in Dairy Cattle from Their Previous Conception Rate and from Their Heredity. J. Dairy Sci., 28: 659-669. 1945.

SERVICE SIRE INFLUENCES ON FERTILITY RATES OF REPEAT BREEDINGS[1]

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Christian and Casida (1) have shown previously from a study of 1,274 second services by ten bulls that some first service bulls influence the conception rates of other bulls used on their repeat service cows. This lowering of fertility was greatest for repeat services when the first service was from semen of a low fertility bull. Salisbury *et al.* (8, 9), using thousands of breedings, have demonstrated that variation among bulls is extensive with respect to differences in delayed returns to service, with age of semen and season of the year also showing measurable differences. Corley *et al.* (3) could demonstrate no real differences in fertility between intrabreed and interbreed inseminations.

The possibility that bulls below average in fertility lower the fertility of their repeat service cows is of considerable economic importance. It is the purpose of this report to present further information on this subject.

DATA AND DISCUSSION

Data from 6,232 inseminations by one technician in one area (Whatcom County) from August 1949 through October 1951 were used for this study. Most of the breedings were made with semen less than 60 hours old, and 90 per cent of the inseminations were known to have been made into the uterus. Penicillin and streptomycin were added irregularly to the semen throughout the period covered. All repeat services were considered, since the inseminator field notes were available for each breeding. Nonreturn estimates were made at successive 30-day intervals in order to determine rates of nonreturn decline. Statistical procedures used were as outlined by Snedecor (11).

The 39 bulls involved in this study were divided into three groups according to 180-day nonreturn rates. Nine bulls were in the low, ten in the high, and 20 in the medium fertility groups. Nonreturn rates were calculated for first services and for repeat services by the same bull and for all other bulls on each bull's repeats. A few instances of crossbreeding occurred, but these were too few to yield significant findings and were deemed unimportant in view of the findings of Christian *et al.* (2). As shown in Table 1, there were no important breed differences in fertility levels or average length of return service interval. The low fertility group (nonreturn rate of 60 per cent or less) consisted of three Guernsey, two Holstein and four Jersey bulls. The range in average fertility between fertility groups was 11 per cent for first service and 13 per cent for repeat services by other sires (Table 1). This difference

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TABLE 1

Effect of fertility level of the bull on the fertility average of repeat services

Breed or group	180-day nonreturn rates						Av. service interval	
	First services		Repeat services					
			Same sire		Other sires			
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(Days)*
Guernsey	2,081	63	189	57	1,171	63	1,348	36.3
Holstein	730	67	67	64	332	61	386	35.5
Jersey	1,023	66	139	60	505	59	643	36.5
Fertility level								
High	1,373	70	153	60	665	65	710	35.6
Medium	1,529	63	121	64	871	63	962	37.6
Low	932	59	112	54	472	52	705	35.0
Total	3,834	64	386	60	2,008	61	2,377	36.2

in range is particularly striking, since repeat services by other sires within breed should have been random with respect to fertility level of the bull. The average fertility of these repeat services by fertility groups should have approximated the repeat service average of 61 per cent instead of ranging from 65 to 52 per cent.

The average length of repeat service interval was highest for the medium fertility group and lowest for the low fertility group. The average for 2,377 known intervals was 36.2 days, with a standard deviation of 26.2 days and a standard error of 0.54 days. There is little likelihood that important differences could be measured between fertility groups in this study. The average service interval compares very closely with that reported by Olds and Seath (7). Christian and Casida (1) reported an average of 32 days, but service intervals longer than 60 days were not considered.

Repeat services with semen from the same bull show little evidence of incompatibility, since 386 services averaged 60 per cent compared with 61 per cent for repeat services with semen from other sires. This finding is in agreement with Christian and Casida (1).

The over-all nonreturn rate for first services in this study was 64 per cent and 61 per cent for repeat services. This difference is less than generally reported, but it may be due to a tendency toward short postpartum service intervals. This is not precisely known, but a study by Erb and Shaw (4) in 1948 revealed that approximately 40 per cent of the cows were being bred sooner than 50 days after calving and that this group of cows was noticeably lower in fertility. This latter point has since been verified by others (5, 6, 10).

In order to elaborate further the results shown for fertility groups in Table 1, the repeats were sorted according to fertility groups by fertility group of subsequent service. As shown in Table 2, the fertility of repeat services by high fertility bulls was 69, 66, and 63 per cent, respectively, when these bulls followed high, medium, and low fertility bulls. Low fertility bulls were 17 per cent below their first service average when bred to repeats of low fertility bulls but only 4 and 5 per cent lower when following medium and high fertility bulls.

TABLE 2

Comparison of fertility of repeat services when bulls of one level follow bulls of other levels of fertility

Group	Following	180-day nonreturn rates[a]		Difference from first service av.
		(No.)	*(%)*	*(%)*
High	high	203	69	—1
High	medium	269	66	—4
High	low	202	63	—7
Medium	medium	484	60	—3
Medium	high	237	68	+5
Medium	low	238	58	—5
Low	low	79	42	—17
Low	medium	181	55	—4
Low	high	115	54	—5

[a] Repeats with the same sire excluded.

Using a low fertility bull on first service cows clearly influenced the fertility of repeat services in this study, even when semen from bulls of high fertility was used for the second service. These results are in substantial agreement with those of Christian and Casida (1).

The high nonreturn rate of 68 per cent for bulls of medium fertility following bulls of high fertility is not readily explained. However, the fertility of bulls of high fertility following bulls of high fertility was 69 per cent. If a fair percentage of first service cows had a short postpartum interval, and the high fertility bulls caused no undesirable after effects, then the return service would occur at a time when the cows were more fertile. Thus, it is possible this deviation is not entirely a random variation.

Twenty-three bulls in this study had in excess of 100 services. This group of bulls was used to determine whether or not important differences existed between fertility groups with respect to decline in nonreturn rate from 30 to 180 days after service. As shown in Table 3, the high fertility bulls had a nonreturn decline of 12 per cent compared with 15 and 17 per cent, respectively, for the medium and low fertility groups. The standard deviation and standard error by bulls by nonreturn interval shows that the bulls on the average showed more similar nonreturn rates at 30 than at 60 days and at 60 than at 90 days.

TABLE 3

Nonreturn decline by groups of bulls of different fertility levels

Fertility level	Bulls	Services	Nonreturn rates					After 180 days	Decline
			30-d.	60-d.	90-d.	120-d.	180-d.		
	(No.)	*(No.)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
High	10	2,191	81	73	70	70	69	69	12
Medium	8	2,432	78	68	64	64	63	63	15
Low	5	1,112	73	63	59	57	56	56	17
Total	23	5,735	78	69	66	65	64	64	14
Standard deviation			4.4	5.6	5.9	5.8	5.8	5.8	
Standard error			0.9	1.2	1.2	1.2	1.2	1.2	

This is also evident in that high fertility bulls had a nonreturn decline of 12 per cent compared with 15 and 17 per cent, respectively, for medium and low fertility bulls.

Results of the analysis of variance showed highly significant differences between fertility groups, between bulls within fertility groups, and for nonreturn decline.

Using individual degrees of freedom, it was found that the difference between medium and high fertility groups was not significant. By the same technique it was found that 120-day nonreturn rates were not significantly higher than 180-day nonreturn rates.

The data were analyzed also by the percentage of returns occurring within 18-24, 25-36, 37-48, 49-80, and after 81 days from previous service by fertility groups using all bulls. There were no significant differences between fertility groups by this method of analysis. The reasons for these findings are obvious in Table 4. This distribution is in excellent agreement with that reported by

TABLE 4

Per cent of returns (distribution) occurring during various service intervals by fertility groups

Fertility group	Repeat services	Days in service interval						
		> 5	5-17	18-24	25-36	37-48	49-80	81+
	(No.)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
High	705	0.6	3.4	51.1	6.8	16.9	13.7	7.5
Medium	1,025	0.1	3.5	51.6	9.3	15.9	13.4	6.2
Low	570	0.0	3.3	50.3	10.5	16.3	13.2	6.3

Olds and Seath (7) for repeat service intervals. The last column of Table 4 appears inconsistent with Table 3. Actually, the two tables are not comparable because of proportionately more repeat services following service by low fertility bulls.

SUMMARY

Data from 6,232 inseminations with semen of 39 bulls by one man in one area of Washington from August 1949 through October 1951 were used for this study. No important breed differences with respect to 180-day nonreturn rates or average repeat service intervals were present. High fertility bulls showed a 180-day nonreturn first service rate of 70 per cent compared with 63 and 59 per cent, respectively, for medium and low fertility bulls. The fertility level of the bull had a marked effect on the fertility of repeat services with semen from other bulls. Low fertility bulls were 17 per cent below their first service average when bred to repeats of low fertility bulls but only 4-5 per cent lower when following medium and high fertility bulls.

The fertility of repeat services by high fertility bulls was —1, —4, and —7 per cent lower, respectively, when they followed bulls of high, medium and low fertility. These results are in agreement with those of Christian and Casida (1), and it is concluded that the low fertility bull lowers the fertility of repeat service cows, even though repeat services are with semen from high fertility bulls.

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REFERENCES

(1) Christian, R. E., and Casida, L. E. The Effects of Infertile Insemination and Individuality of Bulls Upon the Subsequent Fertility of Cows Returning for Service. J. Dairy Sci., **34**: 971-977. 1951.

(2) Christian, R. E., Ulberg, L. C., and Casida, L. E. The Response of Low-fertility Cows to Insemination with Semen from Bulls of Another Breed. J. Dairy Sci., **34**: 988-991. 1951.

(3) Corley, E. L., Bayley, N. D., and Hendrickson, F. A. The Fertility of Interbreed vs. Intrabreed Matings as Determined From Artificial Breeding Data. J. Dairy Sci., **35**: 963-965. 1952.

(4) Erb, R. E., and Shaw, A. O. Breeding Failure Survey in Washington. Proc. 29th Ann. Meeting West Div. A.D.S.A., Corvallis, Ore. 1948.

(5) Moeller, A. N., and VanDemark, N. L. The Relationship of the Interval Between Inseminations to Bovine Fertility. J. Animal Sci., **10**: 988-992. 1951.

(6) Olds, D., Morrison, H. B., and Seath, D. M. Efficiency of Natural Breeding in Dairy Cattle. Ky. Agr. Expt. Sta. Bull. 539. 1949.

(7) Olds, D., and Seath, D. M. Repeatability of the Estrous Cycle Length in Dairy Cows. J. Dairy Sci., **34**: 626-632. 1951.

(8) Salisbury, G. W., Bratton, R. W., and Foote, R. H. The Bull as one Cause of Delayed Returns to Service in Artificial Breeding. J. Dairy Sci., **35**: 250-255. 1952.

(9) Salisbury, G. W., Bratton, R. W., and Foote, R. H. The Effect of Time and Other Factors on the Non-return to Service Estimate of Fertility Level in Artificial Insemination of Cattle. J. Dairy Sci., **35**: 256-260. 1952.

(10) Shannon, F. P., Salisbury, G. W., and VanDemark, N. L. The Fertility of Cows Inseminated at Various Intervals After Calving. J. Animal Sci., **11**: 355-360. 1952.

(11) Snedecor, G. W. *Statistical Methods.* The Iowa State College Press Inc., Ames, 4th ed. 1946.

A STUDY OF ATRETIC LARGE FOLLICLES IN SIX SIRE-GROUPS OF HOLSTEIN-FRIESIAN COWS [1]

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Nymphomania in cows is presumed to result from a deranged relationship between the pituitary and the ovary; the ovarian manifestation of the condition is the presence of follicular cysts. The injection of pituitary extracts will bring about the formation of corpora lutea in cows showing nymphomania, according to Casida *et al.* (3) and Hancock (7). Large cysts, however, may develop in the ovaries without the usual outward manifestations of nymphomania, as stated by Albrechtsen (1) and Garm (5). The difference between cysts (atretic large follicles) which are associated with nymphomania and those that are not is unknown but it is assumed that the latter type represents a hormonal disturbance of a different functional level or kind from that critical for the usual manifestations of nymphomania.

The purpose of the present study was to identify some of the sources of variability in the incidence of atretic large follicles and to evaluate the importance of these structures in the reproductive phenomena of the open cow.

MATERIAL AND METHODS

The data for this report were collected from studies of the experimental herd at the Emmons Blaine Jr. Farm, Lake Mills, Wisconsin. Six lines of breeding built around six unrelated sires are represented in the experimental herd. A "line" as used in this study is composed of the outbred daughters of a particular sire or of the sire and one of his sons. The period covered by this report began October 15, 1947, and included all outbred animals which calved before January 1, 1951, a total of 135 cows and 274 reproductive periods (interval from one parturition to the next in cows or before the first parturition in heifers). The maximum number of reproductive periods for any one animal in the study period was three. The data were collected by rectal palpation of the reproductive organs, as outlined by Kidder *et al.* (8).

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An atretic large follicle is defined as an unruptured follicle which is estimated on palpation to be 25 mm. or more in diameter. Cows or heifers having atretic large follicles may be divided arbitrarily into the following classes on the basis of either reproductive behavior or the stage in the reproductive cycle during which the structures are present:

1. Cows or heifers which show irregular heat periods and bullish behavior in conjunction with atretic large follicles in the ovaries and the absence of corpora lutea (nymphomaniacs).
2. Cows or heifers in which atretic large follicles appear during the regular estrous cycle with corpora lutea present.
3. Cows or heifers which have atretic large follicles during a temporary suspension of the estrous cycle; corpora lutea may be present or absent.
4. Cows which have atretic large follicles during the postpartum anestrous interval, either with or without corpora lutea.
5. Heifers which have atretic large follicles during the prepuberal anestrous period; corpora lutea may be present or absent.

The term "atretic" is used in this report to designate a cow or a reproductive period in which an atretic large follicle was found.

RESULTS AND DISCUSSION

Twenty-seven of 135 animals in this study (20.0%) were "atretic" in at least one of their reproductive periods and one or more atretic large follicles were found in 31 of the 274 reproductive periods (11.3%). The cows in seven of these reproductive periods showed symptoms of nymphomania.

A comparison was made of the proportions of open cows becoming atretic in each month. The highest proportion was found in November (8.2%); no cows became atretic in April or June, and the period in which the incidence of new atretic cows was low extended from April through August. The differences between months were significant when analyzed by analysis of variance technique applicable to enumeration data (4). Differences found are hard to explain because environmental factors are held as constant as possible at the farm. Seasonal differences in light or weather and uncontrolled slight changes in management associated with season are possible causes.

The effect of line on incidence of atretic large follicles was considered over all reproductive periods. Atretic cows were found in all the lines except the L line (Table 1). The highest proportion of atretic reproductive periods was

TABLE 1

Effect of line of breeding on the incidence of atretic large follicles

Line	O	L	H	B	M	N
No. of reproductive periods	55	44	49	49	33	44
% atretic	18.2	0.0	6.1	8.2	12.1	22.7

$X^2 = 15.72$ $P < 0.01$

found in the N line (22.7%). The difference found between percentages of atretic reproductive periods in the different lines was highly significant ($P < 0.01$). This seems consistent with the report on nymphomaniacs by Casida and Chapman (2), in which a heritability figure of 0.43 was given.

The effect of the reproductive period on incidence of atretic large follicles was considered over all lines. The first reproductive period had the lowest incidence (4.6%). The second and third reproductive periods did not differ greatly, having an incidence of 14.8% and 17.5%, respectively. This difference in incidence of atretic large follicles between reproductive periods was significant ($P \cong 0.02$). Atretic large follicles in the first reproductive period were found in only two lines (B and N). They were found in all lines except L in the two other reproductive periods. The low incidence found in the first reproductive period is in accord with various reports on nymphomania. Garm (5) reported a low incidence in the first reproductive period for Swedish Red and Friesian cattle, and Casida and Chapman (2) showed a low incidence in the first reproductive period, but differences in their data did not prove to be statistically significant.

Examination was made to detect a possible critical period after calving when more than a chance proportion of cows developed an atretic large follicle. A comparison was made first of the period before breeding (15-74 days postpartum) and the breeding period (75-300 days). The incidence in the former was 12.0% and in the latter, 4.6%. The difference between these two periods was significant ($P<0.05$). To define the critical period more precisely, the 15-74 day period was divided into four successive 14-day periods (Table 2).

TABLE 2

Effect of postpartum interval on incidence of developing atretic large follicles

Interval postpartum *(days)*	15-29	30-44	45-59	60-74
No. of cows [a]	166	157	149	145
% of cows becoming atretic	6.0	5.1	0.67	0.69

[a] No. of open cows entering the period
$X^2 = 11.95$ $P < 0.01$

The proportion of cows becoming atretic was greatest in the 15-29 day period (6.0%) and the 30-44 day period (5.1%). A highly significant difference was found between the proportions of cows becoming atretic in the four different periods. The high incidence of atretic large follicles immediately after calving is in accord with an observation of Albrechtsen (1) that most cysts develop 14 to 30 days after calving.

Possibly the formation of these atretic large follicles is due to a "rebound" of anterior pituitary function from the inhibition caused by high levels of estrogen present during late pregnancy. Certain experimental work with estrogen has a pertinent relation to this assumption. Hammond and Day (6) point out a latent period in ovarian activity after the removal of stilbestrol

tablets from implanted cows. Hancock (7) compares this latent period with the postpartum anestrous period. Hammond and Day (6) showed that following the latent period produced experimentally by high estrogen levels there was a transient period of follicular cysts. Thus the high incidence of atretic large follicles found in this study may be a sequel to the high levels of estrogen present during late pregnancy.

The repeatability of atretic large follicles was studied by using cows having two successive reproductive periods. Cows were classified (atretic or nonatretic) on the basis of the first of two successive reproductive periods, and their status in the second of two successive reproductive periods then was determined. On this basis seven cows were classified as atretic in the first of two successive reproductive periods, and four of these (57.1%) were atretic in the second reproductive period. One hundred thirty-six cows were classified as nonatretic in the first of two successive reproductive periods, and 17 of these (12.5%) were atretic in the second period. The difference between these percentages was highly significant ($P < 0.01$).

The possibility that high milk-producing ability is associated with a high incidence of atretic large follicles was studied. The milk production of the first lactation was adjusted to a mature-equivalent basis as a measure of inherent producing ability. Cows were arranged in descending order according to their adjusted first lactation record and divided into two equal groups. No significant difference in incidence of atretic large follicles was found between cows with a high inherent producing ability (9.2%) and those with a low inherent producing ability (11.6%) in any reproductive period. This is in accord with work by Casida and Chapman (2) on cystic cows and also with the view of Albrechtsen (1). However, Garm (5) and Williams (9) state that high production increases the incidence of nymphomaniacs.

The effect of the length of interval between parturition and involution of the uterus on incidence of atretic large follicles was studied. The uterus was considered involuted when it attained a fair to good tone and was in or near the pelvic position, and the uterine horns were of approximately equal size. Cows were arranged in descending order according to the length of the interval from parturition to involution of the uterus. They then were divided at the median into two groups. No significant difference in incidence of atretic large follicles was found between the two groups in either reproductive period (17.4% as contrasted with 11.1% in the first reproductive period and 14.3% with 21.4% in the second period). The reproductive periods in which an atretic large follicle appeared did not require a significantly longer time until involution of the uterus (50.0 and 48.1 days) as tested by analysis or variance. Thus, no association between the condition of the uterus and the occurrence of atretic large follicles was found. This differs from the view of Albrechtsen (1), who believed that the development of cysts was due to disease of the uterus. However, Garm (5) states that the condition of the uterus does not affect the incidence of nymphomaniacs.

The incidence of atretic large follicles following a lost pregnancy (5.6%

of 18 cows) did not differ significantly from the incidence following a normal pregnancy (14.4% of 153 cows). The incidence of lost pregnancies following an atretic large follicle (13.6% of 22 cows) was not significantly different from the incidence in nonatretic reproductive periods (6.2% of 226 cows).

The incidence of atretic large follicles in two classes of animals of the different lines is shown in Table 3. Most of the cows in the postpartum anestrous

TABLE 3

Atretic reproductive periods in prepuberal and postpartum anestrous animals of the different lines

Line	O	L	H	B	M	N	Total
Postpartum anestrous cows	6	0	0	1	1	2	10
Prepuberal anestrous heifers	0	0	0	0	0	3	3

class were from the O line and all the prepuberal anestrous heifers were from the N line. The other classes of cows showing atretic large follicles (indicated under *Material and Methods*) were represented fairly evenly throughout the lines.

No treatment for atretic large follicles (including the nymphomaniacs) has been given in the herd. Most cows, however, recovered spontaneously. Twenty-two of the 31 reproductive periods in which an atretic large follicle was found ended in successful pregnancy; four of these were reproductive periods of cows classified as nymphomaniacs. Three reproductive periods ended with cows being slaughtered for reasons other than reproductive trouble; one of these was of a cow which had recovered from nymphomania and in which no atretic large follicles were present at slaughter. The remaining six ended with the cows being slaughtered as nonbreeders. No atretic large follicles were present at slaughter in four of these animals, but they were found in the other two, which were nymphomaniacs.

Although most cows recovered spontaneously from atretic large follicles, the effects of these structures on breeding efficiency must be considered. Breeding efficiency has been measured by the number of days lost between 75 days postpartum and conception. That an atretic large follicle usually increases the number of days lost is shown in Table 4. The magnitude of the effect is not

TABLE 4

Average time lost (in days) by atretic and nonatretic cows in different lines

	Line					
Days lost by	O	L	H	B	M	N
Atretic cows	164	—	105	71	28	133
Atretic with nymphomania [a]	119	—	233	184	6	163
Atretic without nymphomania [a]	169	—	42	33	52	123
Nonatretic cows	64	48	57	51	54	95

[a] Average time lost by all atretic cows with nymphomania was 125 days and for atretic cows without nymphomania, 117 days. This difference was not statistically significant.

uniform, however, and depends upon the line of breeding. This is shown by a highly significant interaction between line and follicular condition ($P < 0.01$).

SUMMARY

The incidence of atretic large follicles was found to be affected significantly by line of breeding, reproductive period, stage of postpartum interval, month, and individuality; it was not found to be affected significantly by inherent producing ability, time from parturition to involution of the uterus, or early loss of pregnancy.

Most cows having atretic large follicles recovered from them without treatment. Breeding efficiency was appreciably lowered in affected cows of most lines because of atretic large follicles, but the magnitude of the response was dependent upon the line of breeding.

REFERENCES

(1) Albrechtsen, J. Sterility in Cattle and Methods of Treatment. Cornell Vet., 7: 57-110. 1917.

(2) Casida, L. E., and Chapman, A. B. Factors Affecting the Incidence of Cystic Ovaries in a Herd of Holstein Cows. J. Dairy Sci., 34: 1200-1205. 1951.

(3) Casida, L. E., McShan, W. H., and Meyer, R. K. Effects of an Unfractionated Pituitary Extract Upon Cystic Ovaries and Nymphomania in Cows. J. Animal Sci., 3: 273-282. 1944.

(4) Chapman, A. B. Personal communication.

(5) Garm, O. A Study on Bovine Nymphomania. Acta. Endocrinol., Suppl. 3, 114 pp. 1949.

(6) Hammond, J., Jr., and Day, F. T. Oestrogen Treatment of Cattle: Induced Lactation and Other Effects. J. Endocrinol., 4: 53-82. 1944.

(7) Hancock, J. L. The Clinical Analysis of Reproductive Failure in Cattle. Vet. Record, 60: 513-517. 1948.

(8) Kidder, H. E., Barrett, G. R., and Casida, L. E. A Study of Ovulations in Six Families of Holstein-Friesians. J. Dairy Sci., 35: 436-444. 1952.

(9) Williams, W. L., and Williams, W. W. Nymphomania of the Cow. North Am. Veterinarian, 4: 232-241. 1923.

REFRACTORINESS OF COWS REPEATEDLY SUPEROVULATED WITH GONADOTROPHINS

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Superovulation in the bovine has been studied by Brock and Rowson (1) and other workers previously cited (15). Egg transplantation in this species has been attempted also (1, 4, 11, 13), and three calves have been produced in this way (14). If these techniques with improvements are to be practical as a tool for breeding dairy cattle by increasing the number of offspring from cows, it will be necessary to superovulate outstanding individuals repeatedly. The purpose of this paper is to describe the response of the ovaries of cows to repeated superovulation by administration of gonadotrophins.

EXPERIMENTAL

Each treatment consisted of either *(a)* five daily subcutaneous injections of sheep (SFSH) or hog (HFSH) pituitary follicle stimulating hormone followed on the 6th day by an intravenous injection of unfractionated sheep pituitary gonadotrophin (USG) or *(b)* one subcutaneous injection of equine gonadotrophin (PMS) followed 5 days later by an intravenous injection of human chorionic gonadotrophin (HCG) or unfractionated sheep gonadotrophin. Each treatment was started on the 15th or 16th day of the cycle. Dosages are given in Table 1. The same dosages of the same preparations were given in the repeated treatments for each cow until refractoriness developed. Then either long periods varying from 133 to 231 days without treatments were allowed or increased dosages were administered to determine whether such procedures would restore some degree of responsiveness.

The sheep pituitary FSH was prepared by slight modification of the method of McShan and Meyer (9). This change consisted of extraction in water adjusted to pH 9.5 with calcium hydroxide. Most of the unfractionated sheep gonadotrophin was prepared by the method developed by these workers (10). These preparations had properties similar to those made in the same manner and used in work reported earlier (15). The HCG, HFSH, PMS, and some of the USG were from commercial sources.

The response to gonadotrophins was measured by counting corpora lutea. The surgery required to do this was facilitated by placing the cow in a specially designed, narrow stall equipped with hoists and rope slings which aided in restraining her. The pelvic viscera were anesthetized by inserting a 2-in., 18-gauge needle at the midline, downward and slightly forward between the

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sacrum and first coccygeal vertebrae or between the first and second coccygeal vertebrae and injecting procaine into the epidural space (7). Twelve ml. of 2% procaine with 1:5,000 epinephrine proved to be the most satisfactory amount for most cows. Then an incision was made with a No. 12 Becton, Dickinson scalpel blade through the dorsal wall of the vagina immediately posterior to the cervix and through the peritoneum of the recto-vaginal pouch. MacDonald (8) recently has described a similar surgical approach to the reproductive organs of the cow. The hand was inserted through the incision, and the ovaries, one at a time, were picked up and pulled into the vagina. The corpora lutea were counted with the aid of a speculum and a head lamp. By turning each ovary in the hand, all surfaces could be seen. Essentially complete asepsis was practiced. In a few of the earliest operations the incision and surrounding areas, including those inside the abdomen and in the vagina, were powdered with sulfanilamide. In later work the areas were rinsed with penicillin or streptomycin in physiological saline solution. Suturing of the vaginal wall was not necessary. Adhesions developed after a varying number of operations and were the principal reason for some of the cows receiving a small number of treatments.

Fifteen grade Holstein and Guernsey cows have been superovulated two or more times. Most of the cows were purchased from cattle buyers. All apparently were normal. The cows whose histories were known had been free of breeding troubles. Only parous cows were selected because of the spaciousness inside their vaginas and vulvas. Most heifers provide insufficient room for the forearm and speculum but probably could be utilized in such work if episiotomies were performed (6).

RESULTS AND DISCUSSION

The sources and dosages of gonadotrophins and number of corpora lutea resulting from successive treatments of individual cows are given in Table 1. In general, the number of corpora lutea decreased with successive superovulations. This trend is clearly shown in Table 2, where averages are given for number of corpora with successive superovulations of cows with at least two, at least three, and at least four treatments. No data for number of corpora lutea for first treatments for cows 35, 37, and 38 were available because these cows were the first in the experiment, and incomplete counts were made. The differences between the means for the twelve cows with two treatments, for the eleven cows with three treatments, and for the nine cows with four treatments are all highly significant ($P < 0.01$).

As can be seen in Table 1, cows 26, 56, 81, 86, and 101 were allowed long periods without treatment after they became comparatively unresponsive. Of these, only cows 81 and 86 showed evidence of regained responsiveness to gonadotrophins. A second rest period of 133 days for cow 81 failed to maintain this recovery. There is a possibility that unresponsiveness (in terms of corpora lutea) of cow 86 to the second treatment was caused by faulty intravenous injection, for her ovaries contained many unruptured follicles and no corpora

TABLE 1

Sources of gonadotrophins, dosages, and number of corpora lutea with repeated superovulation of individual cows

Cow No.	Subcutaneous		Intravenous		No. of corpora lutea from successive treatments					
	Source of FSH	Dose	Source of LH	Dose	1st	2nd	3rd	4th	5th	6th
23	SFSH10[a]	30 g. e.[b]	USG[c]	1 g. e.	19	4 (28)[d]	5 (96)	1 (41)		
26	SFSH10	30 g. e.	USG	1 g. e.	5	1 (62)	14 (62)	2 (231)		
35	SFSH10	30 g. e.	USG	1 g. e.	—	7 (50)	3 (44)	2 (44)		
	SFSH10	50 g. e.	USG	1 g. e.					5 (46)	2 (88)
37	SFSH10	30 g. e.	USG	1 g. e.	—	5 (43)	3 (44)			
38	SFSH10	30 g. e.	USG	1 g. e.	—	5 (93)	11 (18)	1 (44)		
	SFSH10	50 g. e.	USG	1 g. e.					5 (56)	
76	SFSH19	30 g. e.	USG	1 g. e.	21	11 (80)				
98	SFSH19	60 g. e.	USG-A[e]	60 mg.	24	10 (65)	10 (64)	14 (64)		
54	PMS-U[f]	3,000 IU	USG	1 g. e.	40		7 (60)			
	SFSH10	30 g. e.	USG	1 g. e.		14 (45)				
55	PMS-U	3,000 IU	HCG-L[g]	5,000 IU	2	1 (60)	1 (42)	0 (81)		
56	PMS-U	2,000 IU	HCG-L	5,000 IU	2	3 (92)	2 (86)	0 (218)		
57	PMS-U	2,000 IU	USG	1 g. e.	6	1 (55)				
	PMS-U	3,000 IU	USG	1 g. e.			1 (43)			
77	PMS-S[h]	10,000 IU	USG	1 g. e.	17	18 (120)	0 (83)	1 (46)		
81	PMS-S	5,000 IU	USG	1 g. e.	4	2 (66)	1 (94)	4 (223)	1 (133)	
86	HFSH[i]	100 AU[j]	USG-A	60 mg.	25	0 (39)	12 (169)	1 (61)		
101	HFSH	100 AU	USG-A	60 mg.	18	15 (41)	8 (16)	5 (159)	9 (69)	8 (85)

[a] SFSH 10 — Sheep pituitary FSH prepared by the senior author. The number indicates lot.
[b] g. e. — Gram-equivalents of desiccated pituitary.
[c] USG — Unfractionated sheep gonadotrophin prepared by the senior author.
[d] Figures in parentheses are number of days from previous treatments.
[e] USG-A — Unfractionated sheep gonadotrophin — Armour.
[f] PMS-U — Pregnant mare serum — Upjohn.
[g] HCG-L — Human chorionic gonadotrophin — Lakeside.
[h] PMS-S — Pregnant mare serum — Schering.
[i] HFSH — Hog pituitary FSH — Armour.
[j] AU — Armour units.

TABLE 2

Average number of corpora lutea with successive superovulations of all cows with at least 2, at least 3, and at least 4 treatments

Cow No.	Average number of corpora with successive treatments			
	1st	2nd	3rd	4th
12	15.2	6.7		
11	14.7	6.3	5.5	
9	12.9	6.0	5.9	3.1

lutea. The increased response to the third treatment, which followed the second by 169 days, therefore, possibly was due to incomplete refractoriness. The data in general indicate that long periods without gonadotrophin administration is ineffective in overcoming refractoriness.

The dosages of FSH for cows 35, 38, and 57 were increased after the cows had become refractory. Cow 35 showed an increased response, but after a second high dose the number of corpora returned to the low number obtained with the last low dose. Cow 38 showed an increased response with increased dosage, but cow 57 did not. These limited data suggest that the refractoriness can be partially but temporarily overcome by increased dosage.

In the second treatment cow 54 was given SFSH by mistake instead of PMS. The resulting alteration of species as a source of FSH did not prevent the downward trend in number of corpora with successive treatments.

The most probable explanations for the development of refractoriness in the cows in the experiments reported in this paper are the development of antisubstance to gonadotrophin (12, 16), exhaustion of primary follicles in the ovaries, or varying degrees of damage to the ovaries as a result of the treatments or of surgery. The increased responses to increased dosages with cows 35 and 38 might suggest antigonadotrophins as an explanation. The progressive decline in number of corpora lutea in cow 54 in spite of alternating species as sources of FSH would indicate the contrary. The inability of cows 26, 56, 81, and 101 to overcome refractoriness completely by long periods of rest also suggests that antigonadotrophins were not the cause of the refractoriness. Thompson (12) and Zondek and Sulman (16) cite evidence indicating that antigonadotrophic activity in blood serum is usually lost 1 or 2 months after termination of injection of the hormone. Casida *et al.* (2) found only slight evidence of antihormone in serum from cows given large daily doses of unfractionated sheep pituitary extract for 35 or 40 days. During these prolonged treatments the ovaries continued to respond to hormone stimulation and became approximately 60 times the normal size. This work indicated that the production of antigonadotrophin in cows is extremely difficult. Since the treatments given by Casida *et al.* were more prolonged as to period of continuous administration than were the treatments used in our work on superovulation, it does not seem likely that the refractoriness was due to the development of antigonadotrophins.

Further support for this conclusion was obtained by assaying the blood serum from some of the refractory cows for antigonadotrophin. Assay was

done as previously reported (3) using 21-day-old Sprague-Dawley female rats. The serum actually augmented the ovarian response of the rats to gonadotrophins instead of inhibiting it. Similar augmentation was observed with serum from cows prior to administration of gonadotrophins.

Cow 101 was superovulated six times. The number of corpora with the third to sixth treatments, inclusive, was lower than with the first two but showed no continued downward trend. This cow appeared to be older than any others in these studies. Thompson (12) and Zondek and Sulman (16) cite evidence with other species that age can influence the production of antihormone. Cow 98 was another exceptional animal in that she continued to produce a sizable number of corpora through four treatments. In no case was anaphylaxis (5) observed in cows in these experiments.

More work is needed to determine the cause of the apparent refractoriness of cows to gonadotrophins administered for the purpose of producing repeated superovulation. Research with this objective in mind is being continued.

SUMMARY AND CONCLUSIONS

Fifteen cows were superovulated repeatedly two to six times. The different sources of gonadotrophins included sheep pituitary FSH, unfractionated sheep pituitary gonadotrophin, equine gonadotrophin, human chorionic gonadotrophin, and hog pituitary FSH.

In general, there was a decline in number of corpora lutea with successive superovulations. The differences between the means for the twelve cows with two treatments, for the eleven cows with three treatments, and for the nine cows with four treatments were highly significant ($P < 0.01$).

Long periods without administration of gonadotrophins failed to eliminate the refractoriness. Limited data suggest that increased dosage partially but only temporarily overcame the refractoriness.

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REFERENCES

(1) Brock, Helen, and Rowson, L. E. The Production of Viable Bovine Ova. J. Agr. Sci., 42: 479-482. 1952.

(2) Casida, L. E., Meyer, R. K., McShan, W. H., and Wisnicky, W. Effects of Pituitary Gonadotropins on the Ovaries and the Induction of Superfecundity in Cattle. Am. J. Vet. Research, 4: 76-94. 1943.

(3) Deutsch, H. F., McShan, W. H., Ely, C. A., and Meyer, R. K. Time of Appearance and Properties of Antigonadotrophic and Progonadotrophic Substances of Rabbit Serum. Am. J. Physiol., 162: 393-405. 1950.

(4) Dowling, D. F. Problems of the Transplantation of Fertilized Ova. J. Agr. Sci., 39: 374-396. 1949.

(5) Dragstedt, C. A. Anaphylaxis. Physiol. Revs., 21: 563-587. 1941.

(6) FREIERMUTH, G. J. Episiotomy in Veterinary Obstetrics. J. Am. Vet. Med. Assoc., 113: 231-232. 1948.

(7) GUARD, W. F. *Surgical Technics for Veterinary Students.* Edwards Bros., Inc., Ann Arbor, Mich. 1948.

(8) MACDONALD, L. E. Vaginal Approach for Removal of the Corpus Luteum. J. Am. Vet. Med. Assoc., 120: 128-129. 1952.

(9) MCSHAN, W. H., AND MEYER, R. K. The Preparation and Properties of Pituitary Follicle-stimulating Fractions Made by Trypsin Digestion. J. Biol. Chem., 135: 473-482. 1940.

(10) MCSHAN, W. H., AND MEYER, R. K. Preparation of Sheep Pituitary Gonadotropin and Recovery of the Lactogenic Hormone. J. Biol. Chem., 151: 259-266. 1943.

(11) ROWSON, L. E. Methods of Inducing Multiple Ovulation in Cattle. J. Endocrinol., 7: 260-270. 1951.

(12) THOMPSON, K. W. Antihormones. Physiol. Revs., 21: 588-631. 1941.

(13) UMBAUGH, R. E. Superovulation and Ovum Transfer in Cattle. Am. J. Vet. Research, 10: 295-305. 1949.

(14) WILLETT, E. L., BUCKNER, P. J., AND LARSON, G. L. Three Successful Transplantations of Fertilized Bovine Eggs. J. Dairy Sci., 36: 520-523. 1953.

(15) WILLETT, E. L., MCSHAN, W. H., AND MEYER, R. K. Relation of Stage of Cycle and Source of Luteinizing Hormone to Superovulation in Dairy Cattle. Proc. Soc. Expt. Biol. Med., 79: 396-400. 1952.

(16) ZONDEK, B., AND SULMAN, F. *The Antigonadotropic Factor with Consideration of the Antihormone Problem.* Williams and Wilkins Co., Baltimore, Md. 1942.

A STUDY OF THE EFFECT OF STILBESTROL INDUCED LACTATION ON DAIRY GOATS

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During the first two decades of this century the discovery of hormones and the development of the hormone theory in its relation to the physiological processes of animal growth and reproduction led to considerable study of the growth of the mammary glands and the development of their duct systems. The early work was done with small laboratory animals, including guinea pigs, mice, rats, rabbits, cats, and dogs.

Apparently de Fremery (2) was first to make use of estrogens to bring goats into lactation. Since this time several investigators have examined the efficiency of various estrogenic preparations applied by injection, implantation, or inunction for inducing lactation in young unbred females. Among those who have worked with goats are Folley *et al.* (4, 5), Folley and Young (6), Lewis and Turner (8, 9), Lewis (7), Mixner and Turner (11), Folley, Malpress, and Young (3), and Meites and Turner (10). All have shown that lactation may be induced in virgin females and in dry goats by the use of synthetic estrogenic preparations.

PLAN AND PURPOSE OF EXPERIMENT

In 1947 the Bureau of Animal Industry in cooperation with the Bureau of Dairy Industry started an experiment with the milking goat herd of the Bureau of Animal Industry. The primary purpose of the experiment was to identify at the earliest possible age female goats having a superior capacity for milk production. The animals used were purebred or high grade Toggenburgs. The female kids which were born each spring (February to April) were divided into two groups, one group to be treated with stilbestrol, the other untreated. The animals born in the five different years of the experiment were designated as series A - E, the induced lactations occurring 1947-1951, respectively. No control does were maintained for the E series. Treatment was begun in May when the kids were 13 to 16 months of age. Subcutaneous injections of 0.5 mg. of diethylstilbestrol in 1 ml. of oil were administered three times per week for a period of about 50 days. Milking began as soon as fluid was present in the udder. For the first 2 years of the experiment twice-a-day milking began when the daily milk production reached about 1 lb. In the later years once-a-day milking was resorted to because of labor shortage. Milking

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continued until the daily production became less than 1 lb. In the fall, animals were checked daily for signs of estrus and bred when estrus was evident.

RESULTS

Several goats began secreting a few milliliters of milk after the fourth or fifth injection. This gradually increased in volume so that by the end of 3 to 4 weeks, daily milking was begun with 1 lb. or more daily production. This increased rather rapidly to 2.5 to 3.0 lb. per day for the best producers during their peak production. Daily production then gradually decreased until it fell below 1 lb. per day, and daily milking was discontinued. The best producers were in lactation 5 to 6 months. Lactation curves for the induced lactation resembled those for normal lactation except that the production was at a lower level and did not continue for as long a period. Other does responded more slowly, not showing secretion for perhaps a month after injections were started. Daily production did not rise much above 1 lb. and lactation persisted for only 3 to 4 months. Other does secreted a few milliliters of milk for only five or six times or did not react at all to the injections.

Thus, in the injected group the production for induced lactations ranged from no response to over 360 lb. of milk. The duration of the induced lactations extended up to 185 days. For their first natural lactation the injected group produced from 3 lb. to over 1,500 lb. of milk. Length of lactation ranged from 10 to 419 days. Production during the first lactation of the does of the control group ranged from 127 to over 1,600 lb. and duration of lactation ranged from 118 to 306 days. Animals which died before a lactation was completed are not included in these production ranges. For the most part, animals which responded well in their induced lactation also did well in their natural lactation, and those which gave a poor response in their induced lactation were poor producers in their natural lactations. These relationships may be seen in Table 1.

Several correlations were calculated to show the closeness of relationships between induced and natural lactation and to determine what method of com-

TABLE 1

Production levels of Stilbestrol-injected yearling does

Induced lactation production level	No natural lactation	First natural lactation production level					Died	Total
		0-100 lb.	101-500 lb.	501-1000 lb.	1001-1500 lb.	1501- lb.		
(lb.)	*(No.)*	*(No.)*	*(No.)*	*(No.)*	*(No.)*	*(No.)*	*(No.)*	*(No.)*
0-50	9	2	1	4	....	....	2	18
51-100	2	....	1	2	1	1	2	9
101-300	3	....	....	5	2	....	1	11
301-500	1	....	....	1	1	....	....	3
Total	15[a]	2	2	12	4[b]	1[b]	5	41

[a] Twelve of these failed to breed; the 3 others bred, but aborted before term and were not rebred.

[b] Besides these 5 which produced in excess of 1,000 lb. during their first lactation, 4 others produced more than 1,000 lb. in their second lactation, 3 of which produced more than 200 lb. in their induced lactation.

parison would give the highest prediction for future milk yields based on the performance of induced lactation. The correlations calculated, with number of degrees of freedom, are shown in Table 2. Only animals which had one or

TABLE 2

Correlations of milk yield during induced and natural lactations

Factors correlated	Degrees of freedom	Correlation value
Induced daily peak production with daily peak of first natural lactation	23	0.727[a]
Induced daily peak production with total of first natural lactation	19	0.718[a]
Total induced production with total production of all natural lactations	25	0.682[a]
Induced daily peak production with total production of all natural lactations	25	0.686[a]
Induced daily average production with average daily production of first natural lactation	23	0.623[a]
Total induced lactation with total production of first natural lactation	19	0.572[a]
Induced lactation by 30-day periods with corresponding 30-day periods of first natural lactation	61	0.456[a]
No. of days of induced lactation with no. of days of first natural lactation	19	0.506[b]

[a] Highly significant P = 0.01
[b] Significant P = 0.05

more complete lactations were used in calculating the correlations; those which died during the lactation period were discarded, except in two correlations where daily average and daily peak production were used (those with 23 degrees of freedom). Several of these correlations are fairly high and all are significant. Figure 1 shows graphically the relationship between induced daily peak production, total induced production, and total first natural production.

The average yearly production per doe for the first natural lactation of the stilbestrol-injected group was compared with the first lactation of the controls and found to differ by only 67.4 lb. Number of days in lactation per doe for the two groups differed by 23. Neither difference was significant. For the second natural lactation the average difference in yearly milk production per doe between injected and control does was less than 1 lb. and the average difference in duration of lactation about 6 days. These differences also were not significant.

The average daily production for the first natural lactation of the stilbestrol-injected animals was compared with that of their controls and found to differ by only 0.04 lb. per doe. The corresponding difference for second natural lactation was 0.1 lb. Neither of these differences was significant.

It was noted that some of the animals of both the injected and the uninjected groups failed to come into estrus or to conceive in the fall following their in-

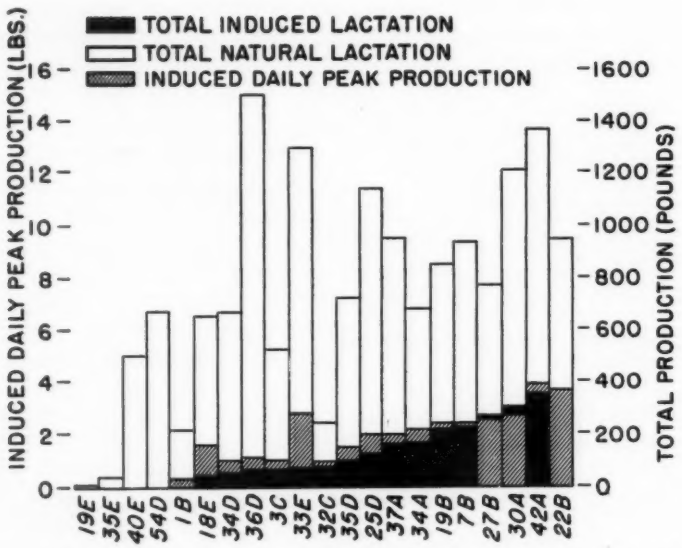

FIG. 1. Relation between total induced lactation, total first natural lactation, and induced daily peak production of stilbestrol-injected does.

jection and induced lactation. Analysis was made to determine whether or not there were significant differences between the two groups. For the first breeding period, the number of animals injected, the number and percentage which

TABLE 3

Breeding performance of yearling does subsequent to stilbestrol injections as compared to uninjected controls

Year and Series		Treated				Untreated			
		No.	Showed estrus	Bred	Not in estrus	No.	Showed estrus	Bred	Not in estrus
1947 A	(No.)[a]	7	6	4	1	10	9	7	1
	(%)		85.7	66.7	14.3		90.0	77.8	10.0
1948 B	(No.)	8	8	7	0	8	8	4	0
	(%)		100.0	87.5	0		100.0	50.0	0
1949 C	(No.)	5	5	4	0	5	5	3	0
	(%)		100.0	80.0	0		100.0	60.0	0
1950 D	(No.)	12	12	8	0	10	10	8	0
	(%)		100.0	66.7	0		100.0	80.0	0
1951 E	(No.)	9	7	7	2	No control group			
	(%)		77.8	100.0	22.2				
Total	(No.)	41	38	30	3	33	32	22	1
	(%)		92.7	78.9	7.3		97.0	68.7	3.0

[a] Percentages showing estrus and not in estrus are calculated on total number of animals in the experiment at time of first fall breeding season following induced lactation. Percentage bred is calculated from the number which showed estrus.

showed estrus and bred, and the number which failed to show estrus are shown in Table 3. There was no statistically significant difference between the injected and uninjected groups in number failing to show estrus or in number failing to breed of those which showed estrus.

After the experiment had been in progress for 3 or 4 years, it was noticed that some of the injected animals had failed to come into estrus or breed, not only as yearlings, but in subsequent breeding seasons. It was thought that possibly permanent injury had been done to the animals by their induced lactation. However, comparison of the injected and uninjected groups for all of their breeding seasons failed to show significant differences in their estrus or breeding records. This is shown in Table 4.

Only three does of the injected group failed to breed. Doe 11 A never

TABLE 4

Breeding record of stilbestrol injected does and their controls in first and subsequent breeding seasons

Breeding season	Injected				Control			
	No.	In estrus	Bred	Not in estrus	No.	In estrus	Bred	Not in estrus
Series A								
1947	7	6	4	1	10	9	7	1
1948	6	5	5	1	6	6	6	0
1949	6	5	3	1	6	6	5	0
1950	3	3	2	0	4	2	1	2
1951	1	1	1	0	2	1	0	1
Total No.	23	20	15	3	28	24	19	4
(%)[a]		87.0	75.0	13.0		85.7	79.2	14.3
Series B								
1948	8	8	7	0	8	8	4	0
1949	5	5	4	0	8	8	8	0
1950	2	2	2	0	6	6	6	0
1951	1	1	1	0	5	4	4	1
Total No.	16	16	14	0	27	26	22	1
(%)		100.0	87.5	0		96.3	84.6	3.7
Series C								
1949	5	5	4	0	5	5	3	0
1950	3	2	2	1	2	2	1	0
1951	2	2	2	0	1	1	1	0
Total No.	10	9	8	1	8	8	5	0
(%)		90.0	88.9	10.0		100.0	62.5	0
Series D								
1950	12	12	8	0	10	10	8	0
1951	9	8	8	1	5	5	3	0
Total No.	21	20	16	1	15	15	11	0
(%)		95.2	80.0	4.8		100.0	73.3	0
Series E								
1951	9	7	7	2	[b]			
(%)		77.8	100.0	22.2				
Grand Totals								
No.	79	72	60	7	78	73	57	5
(%)		91.1	83.3	8.9		93.6	78.1	6.4

[a] Percentages showing estrus and not in estrus are calculated on total number of animals in the experiment. Percentage bred is calculated on the number showing estrus.

[b] There was no control group for series E.

showed estrus and at slaughter when 46 months of age was found to have a cystic left ovary, an atrophied right ovary, and an imperforate uterus. Does 9 E and 30 E did not come into estrus in the fall after their injection with stilbestrol and were injected December 11, 1951, with P.M.S. Neither responded. Both were injected in April 1952 with E.C.P. (estradiol cyclopentylpropionate, Upjohn), came into estrus, but failed to conceive. Both came into estrus again in the fall of 1952. One kidded in February, the other in April, 1953.

DISCUSSION

The results of this experiment agree with most others of a similar nature. Other investigators also have found that individual response to hormone injections and the production level of those which do respond is highly variable (Cowie *et al.*, 1). Most goats which gave a satisfactory response came into lactation promptly after the first few injections and reached their highest daily average by the second or third month. However, some did not reach full production until much later, and production dropped off rather suddenly. Those which were the poorest producers yielded only a few milliliters after varying intervals, seldom producing enough for daily milking. Some did not respond at all. Some of the uninjected does of groups B and C came into lactation. Most of them produced only a few milliliters for a few days, but two in group B milked for more than a month, one averaging 0.88, the other 0.51 lb. per day. Turner and Gomez (12) and Folley *et al.* (5), as well as practical goat dairymen, have observed this to be a frequent occurrence in goats.

Induced lactation curves of those which responded favorably to stilbestrol injection resembled closely those of the first natural lactation. It is not clear why series C, D, and E, did not respond as favorably to injection of stilbestrol as did series A and B. Lower levels of production and shorter lactation periods may be accounted for in part by once-a-day milking. Poor quality roughage at the period when series C, D, and E were in lactation also may account in part for the poor showing of these groups. That the injected and uninjected groups were similar as regards potential milk producing capacity was shown by the small differences between the two groups for their first and second natural lactations, not only in production level but also in duration of lactation.

Of the several correlations calculated between induced and natural lactations, it is desirable to determine which would be the best criterion to use as a basis for culling or to predict future milk production performance. Daily peak production or average daily production is valuable only if production levels are maintained for a considerable period of time. The correlations in which both production level and duration of lactation enter are: induced daily peak production correlated with total production for all lactations, total induced production correlated with total production for all lactations, induced peak daily production correlated with total production for the first natural lactation, and total induced lactation correlated with total production of the first natural lactation. The first three of these correlations approximate 0.7 in value and are highly significant. It can be concluded from these three highly significant values that

culling would be fairly safe on this basis. Extremely high and extremely low producers probably could be identified with a high degree of certainty. A few medium producers which probably would be worth retaining in a herd might be overlooked. Whether the extra work of injecting and milking the yearlings would be offset by the ability to cull before the fall breeding and thus save a year's feeding of potentially low producers has not been considered in this experiment.

Data failed to show any harmful effect of the injections on the subsequent breeding performance of the animals. Actually a higher percentage of the injected group bred than of the control group, although the difference was not significant.

SUMMARY AND CONCLUSIONS

Virgin yearling does were divided into two groups. One group was injected subcutaneously three times a week with 0.5 mg. diethylstilbestrol for a period of about 50 days. The other animals were uninjected and were used as controls. Milking was begun as soon as a secretion was present, and when the secretion reached approximately 1 lb., twice-a-day milking was practiced.

Some of the injected does came into milking by the fourth or fifth injection, and the milk gradually increased in volume until by the end of the second month daily production was as high as 3 lb. or more for the best milkers. Other injected does either were slow in coming into milk or gave no response to the injections. Those which did milk had short lactations with low production. A few does milked without injections. The induced lactation curves were similar to natural lactation curves.

For the most part, the animals which were good producers under induced lactation proved to be good producers in their natural lactations. In general, it should be expected that animals which produce 100 lb. or more of milk in an induced lactation will produce about 1,000 lb. in their first natural lactation. The correlations between induced lactation and first natural lactation exceeded 0.6 when based on three different criteria, and all were highly significant.

The injected and uninjected animals were alike so far as their potential milking capacity was concerned, as determined by their first and second natural lactations.

Injection and prebreeding lactation had no harmful effect on future estrus and breeding. A slightly higher percentage of the noninjected group showed estrus, but a higher percentage of the injected group bred. The differences between the groups were not significant.

REFERENCES

(1) Cowie, A. T., Folley, S. J., Malpress, F. H., and Richardson, K. C. Studies on the Hormonal Induction of Mammary Growth and Lactation in the Goat. J. Endocrinol., **8**: 64-88. 1952.

(2) de Fremery, P. On the Influence of Different Hormones on Lactation. Proc. Physiol. Soc., J. Physiol., **87**: 50-51. 1936.

(3) FOLLEY, S. J., MALPRESS, F. H., AND YOUNG, F. G. Induction of Lactation in Goats and Cows with Synthetic Oestrogens and Anterior Pituitary Extracts. J. Endocrinol., 4, 1: 181-193. 1944.

(4) FOLLEY, S. J., WATSON, HELEN M. SCOTT, AND BOTTOMLEY, A. C. Induction of Lactation in Goats with Diethylstilbestrol Dipropionate. Proc. Physiol. Soc., J. Physiol., 98: 15. 1940.

(5) FOLLEY, S. J., WATSON, HELEN M. SCOTT, AND BOTTOMLEY, A. C. Studies on Experimental Teat and Mammary Development and Lactation in the Goat. J. Dairy Research, 12, 3: 241-264. 1941.

(6) FOLLEY, S. J., AND YOUNG, F. G. Artificial Induction of Lactation in Virgin Animals. Nature 148, 3758: 563-4. 1941.

(7) LEWIS, A. A. Artificial Freshening of Goats. Dairy Goat J., 20: 4-6. 1942.

(8) LEWIS, A. A., AND TURNER, C. W. Effect of Stilbestrol on the Mammary Gland. Proc. Am. Soc. Animal Production, 63-66. 1940.

(9) LEWIS, A. A., AND TURNER, C. W. Effect of Stilbestrol on the Mammary Gland of the Mouse, Rat, Rabbit and Goat. J. Dairy Sci., 24: 845-860. 1941.

(10) MEITES, JOSEPH, AND TURNER, C. W. The Experimental Induction and Maintenance of Lactation in Goats with Estrogen-Thyroid Combinations. Proc. Am. Soc. Animal Production, J. Animal Sci., 6, 4: 499. 1947.

(11) MIXNER, JOHN P., AND TURNER, C. W. The Mammogenic Hormones of the Anterior Pituitary. II. The Lobule-Alveolar Growth Factor. Mo. Agr. Expt. Sta. Research Bull. 378. 1943.

(12) TURNER, C. W., AND GOMEZ, E. T. The Development of the Mammary Glands of the Goat. Mo. Agr. Expt. Sta. Research Bull. 240. 1936.

THE OCCURRENCE OF BACTERIA RESISTANT TO PENICILLIN, STREPTOMYCIN, AND SULFANILAMIDE IN DILUTED BULL SEMEN [1]

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The effectiveness of the addition of antibacterial agents in increasing the fertility of bovine semen has been demonstrated by several workers (1, 8, 9, 10, 17), and many others (2, 4, 6, 8, 11, 12, 15) have shown a marked decrease in the bacterial count of diluted semen containing antibiotics added either singly or in combination. Most of these investigators have reported counts of less than 300 bacteria per milliliter in diluted semen containing 500-1000 units of penicillin and/or 500-1000 γ of streptomycin per milliliter. The most commonly employed medium has been veal or beef infusion agar (2, 4, 11, 12, 15), although tryptone glucose extract agar (19) and blood agar (3, 6, 8) have been used. Although it is known that the action of the sulfonamides and antibiotics is often bacteriostatic rather than bactericidal, in none of the previous investigations was there any substance added at the time of plating to specifically inactivate the antibacterial agents in the diluted semen. Unless the dilution of the sample was sufficiently great to decrease the concentration of the antibacterial agent to a noninhibitory level in the petri dish, the counts that were observed may have been too low. Plastridge and Easterbrooks (16) in a study of the effect of antibiotics on *Vibrio fetus* found that streptomycin in a concentration of less than 300 γ per milliliter of diluent did not kill the bacterium, although it was prevented from growing by a concentration of 1.0 γ per milliliter. Janeway (13) suggested that para aminobenzoic acid (PABA) be incorporated in media used to cultivate bacteria from the blood of patients receiving sulfonamides. After the studies of McQuarrie and Leibmann on penicillinase (14), this enzyme was recommended for use in blood cultures from patients undergoing penicillin therapy (18). With the increased use of a combination of sulfanilamide, penicillin, and streptomycin in diluted semen, it was considered advisable to determine whether or not inactivation of these substances would reveal higher counts of bacteria in the semen than had heretofore been recognized. If such organisms are present in the semen, their relation to fertility should be determined, since the inhibitory action of the antibacterial agents probably would be removed within the reproductive tract of the cow in a short while both by absorption of the substance and by dilution. This would leave any microorganism introduced with the semen free to grow.

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EXPERIMENTAL METHODS

All samples of semen were obtained from the Mississippi Artificial Breeders Cooperative. The semen was extended with egg yolk-citrate diluter containing 3 mg. per milliliter of sulfanilamide, 500 units per milliliter of penicillin, and 500 γ per milliliter of streptomycin. The rate of dilution varied from 1:15 to 1:70, depending upon the demand for semen from the individual bull. The semen was most frequently distributed at a dilution of 1:30; therefore, all bacterial counts were adjusted to that dilution. Since most of the semen was used for insemination within 24-36 hours after dilution, the samples were held at 5° C. for 24 hours before plating. A few samples were held an additional 48 hours to determine what effect longer exposure to the antibacterial agents would have on the count.

The medium used for this investigation was Difco heart infusion agar containing PABA and penicillinase. Fifty mg. of PABA per 100 ml. was added to the medium before sterilization and 1 ml. of Difco Penase was added per 100 ml. of medium after cooling to 45-50° C. (One ml. of this penicillinase was shown by suitably controlled tests to be able to inactivate between 40,000 and 45,000 units of penicillin). Five per cent defibrinated bovine blood was added to the medium in the early part of the investigation; it was omitted in the later samples after duplicate sets of plates with and without added blood indicated little or no difference in counts or in types of microorganisms.

No enzyme similar to penicillinase has been described which will neutralize the effect of streptomycin. However, it will be neutralized in a strongly reducing medium (5, 7). The following procedure was used in a part of the work in an attempt to neutralize the antibiotics. Difco Thiol medium (a strongly reducing medium rich in SH groups) plus 0.05% PABA and 1.5% agar was prepared according to directions, dispensed in 20-ml. amounts in tubes, and sterilized in an autoclave at 121° C. for 15 minutes. The medium was cooled to 45-50° C., and 0.3 ml. of penicillinase was added to each tube. An aliquot of the semen containing the antibiotics was added to the tube, mixed thoroughly, and allowed to stand at 48° C. for 10 minutes. The medium then was poured into a sterile petri dish. Plastridge and Easterbrooks (16) reported that streptomycin was remarkably stable in Thiol medium. This is contrary to the opinion of the producer, who states that up to 10,000 γ of streptomycin will be inactivated by a tube of Thiol medium (7).

To determine the effect of the antibacterial agents alone and in combination on the bacterial count of semen, raw semen was mixed with egg yolk-citrate diluter, divided into six aliquots, and the usual concentrations of the antibacterial agents added alone and in combination to five of them. The quantity of the diluter added varied according to the amount of semen available. All samples were held at 5° C. for 24 hours before plating, and the counts are recorded on the basis of the undiluted semen.

The plates for total counts were incubated aerobically at 37° C. for 96 hours. Duplicate sets of plates of several of the samples also were incubated in the presence of 10% CO_2 to determine whether or not any microorganisms were

present that required CO_2 for their initial growth. This was discontinued when no differences in count or type of flora could be found. Anaerobic incubation of plates was not used since Coleman (6) had found that there were few strict anaerobes in bovine semen. The resistance of selected strains of diphtheroids to different antibiotics was determined by means of Difco sensitivity disks. The disks were placed on seeded layers of BBL trypticase-soy agar overlaid on sterile agar. The plates were incubated for 72 hours at 37° C. and the zones of inhibition measured with the aid of a Quebec colony counter.

RESULTS AND DISCUSSION

The effect of neutralization of the sulfanilamide and antibiotics in the sample on the total count is shown in Table 1. The heart infusion agar alone did not yield

TABLE 1

Comparison of media for the enumeration of antibiotic-resistant bacteria in diluted semen

Sample No.	Total bacterial plate count per ml. on:		
	H.I. agar	H.I. agar [a] plus PABA and penase	Thiol agar plus PABA and penase
1	110	1,800	2,000
2	30	40	50
3	180	1,500	1,200
4	130	6,000	4,500
5	2,500	2,700	2,400
6		180	360
7		540	390
8		6,600	6,900
9		500	500
10		12,000	9,000
Av.	490	3,200	2,700

[a] Difco heart infusion agar.

counts nearly as high as did the other two media, except in cases (e.g., sample 5) in which the serial dilutions of the extended semen were 1:100 or greater. It was noted in samples with a relatively high count that the plates at the higher dilutions often had more colonies than those at lower dilutions. When tested against a sensitive strain of *Micrococcus pyogenes* var. *aureus* the Thiol agar was found to inactivate at least 500 γ of streptomycin. Since this was contrary to the observations of Plastridige and Easterbrooks using liquid Thiol medium (16), an additional test employing the liquid medium was made. In this test, inactivation of at least 2000 γ of streptomycin was observed. However, the Thiol agar did not yield counts any higher than the heart infusion agar containing PABA and penicillinase. Because of a precipitate that usually formed when the Thiol agar was mixed with the egg yolk-citrate diluter and the more complex procedure involved, the Thiol agar was not used for the remainder of the investigation. In a limited comparison on five samples, BBL trypticase-soy agar gave counts similar to duplicate samples on heart infusion agar.

The ranges of total counts of bacteria resistant to sulfanilamide, penicillin, and streptomycin and the fertility of bulls shedding these bacteria are shown in Table 2. These data indicate that the presence or absence of these resistant

TABLE 2

Range of total counts and fertility of diluted semen containing sulfanilamide, penicillin, and streptomycin

Range of total counts[a]	No. bulls	No. samples	No. first services	60-90 day N.R.
(/ml.)				*(%)*
10-1,000	11	33[b]	893	71.0
100-16,000	7	26[c]	512	71.7

[a] Range for all samples from a single bull.
[b] 28 of these samples had counts of <200/ml.; 1 had a count of >800/ml.
[c] 12 of these samples had counts of >4,000/ml.; 5 had counts of 800–4,000/ml.; 6 had counts <200/ml.

bacteria is apparently not related to the fertility of the semen. Although wide variations in bacterial counts on raw semen have been shown (3), the differences in counts exhibited by these two groups indicate that a bull producing semen with a relatively low count one time will produce a low count semen consistently. For example, the semen from one bull had a count of less than 10 antibiotic-resistant bacteria per milliliter of diluted semen on four samples taken at weekly intervals, whereas another bull produced semen with a count over 10,000 per milliliter on three of four samples. The 60-90 day nonreturns on these two bulls were 74% and 72%, respectively. In all semen samples held for a total of 72 hours at 5° C. and then plated, the counts were similar to the counts obtained after 24 hours.

Table 3 shows the effect of sulfanilamide, penicillin, and streptomycin alone and in combination on the bacterial count of semen. It is apparent from this table that sulfanilamide did not contribute materially to the control of the

TABLE 3

Effect of sulfanilamide, penicillin, and streptomycin alone and in combination on total bacterial counts of semen

Sample No.	Counts[a] on blood agar containing 0.05% PABA and 1% Penase					
	Raw semen	Sulfanilamide (3 mg./ml.)	Penicillin (500 units /ml.)	Streptomycin (500 γ/ml.)	P'cillin & S'mycin (500 units & 500 γ/ml.)	All three
	(Bacteria/ml.) (× 1,000)					
1	1	1	1.2	0.6	0.5	0.3
2	180	81	67	110	39	34
3	700	540	300	320	310	340
4	8	7	0.6	0.6	0.2	0.4
5	37	20	12	10	11	10
Av. of 15 samples	170	120	73	84	68	71

[a] These counts are on basis of undiluted semen.

bacteria in the semen when the antibiotics also were included. Although penicillin or streptomycin when used alone did, in some samples, give counts as low as the combination (samples 1 and 3), these data indicate that neither can be regarded as capable of controlling the bacterial content as effectively as will a combination of the two.

Most of the bacteria surviving in the semen were diphtheroids. A few yeast also were usually present. The resistance of representative strains of the diphtheroids to seven different antibiotics at 37° C. is presented in Table 4.

TABLE 4

Resistance at 37° C. of representative diphtheroids from bull semen to antibiotics

Culture No.	Diameter (mm.) of zone of inhibition around disks containing antibiotic concentrations as follows:													
	streptomycin γ		penicillin units		aureomycin γ		chloromycetin γ		terramycin γ		bacitracin units		polymyxin B γ	
	10	100	1	10	10	30	10	60	10	30	2	10	5	30
7	0	0	0	15	25	27	8	12	25	27	8	14	8	14
43	0	0	0	7	27	27	8	14	25	28	8	15	8	16
106	0	0	0	12	25	30	6	12	25	35	8	17	9	15
108	0	0	0	30	30	33	8	12	25	32	8	14	8	14
134	0	0	0	0	19	21	7	10	9	16	7	9	10	20

These resistant bacteria are most effectively controlled by terramycin and aureomycin. Whether this was a bacteriostatic or bactericidal action was not determined.

The results obtained in this study indicate that there may be several hundred bacteria per milliliter of diluted semen even though it contains streptomycin, penicillin, and sulfanilamide. However, the presence of these bacteria apparently does not affect the fertility of the semen. It is interesting to note that although the growth of these bacteria may be inhibited by as little as 10 units of penicillin, they are not killed by exposure to at least 500 units for as long as 72 hours. The addition of sulfanilamide to diluted semen for its effect on the bacterial content does not appear to be warranted. Its continued inclusion in the diluted semen may be justified on the basis of its effect on the spermatozoa (9), although studies by Foote and Bratton (11) question its usefulness in this respect.

SUMMARY

Bacterial plate counts of over 10,000 per milliliter have been obtained from samples of diluted bull semen containing sulfanilamide, penicillin, and streptomycin. These counts were obtained when penicillinase and para amino-benzoic acid were included in the plating medium to inactivate the penicillin and sulfanilamide. Comparison of per cent nonreturns from semen with low and with relatively high counts indicated that these bacteria were not related to fertility. Sulfanilamide had little or no effect on the bacterial content of semen containing penicillin and streptomycin. The inclusion of both of the antibiotics is desirable,

however, since bacteria are usually present that are resistant to one but not to the other. The resistant bacteria are primarily diphtheroids and are inhibited but not killed by low concentrations of penicillin; however, they are not inhibited by relatively high concentrations of streptomycin.

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REFERENCES

(1) ALMQUIST, J. O. The Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. J. Dairy Sci., 32: 950-954. 1949.

(2) ALMQUIST, J. O., GLANTZ, P. J., AND SHAFFER, H. E. The Effect of a Combination of Penicillin and Streptomycin upon the Livability and Bacterial Content of Bovine Semen. J. Dairy Sci., 32: 183-190. 1949.

(3) ALMQUIST, J. O., PRINCE, P. W., AND REID, J. J. Bacteriological Studies of Bovine Semen. I. Numbers of Bacteria and the Relation to Fertility. J. Dairy Sci., 32: 543-548. 1949.

(4) ALMQUIST, J. O., THORP, W. T. S., AND KNODT, C. B. The Effect of Penicillin upon the Livability, Glycolysis, and Bacterial Content of Bovine Semen. J. Dairy Sci., 31: 11-19. 1948.

(5) BONDI, A., JR., DIETZ, C. C., AND SPAULDING, E. H. Interference with the Antibacterial Action of Streptomycin by Reducing Agents. Science, 103: 399-401. 1946.

(6) COLEMAN, W. D. The Bacterial Flora of Semen from Bulls of High and Low Fertility. M.S. thesis. La. State Univ. 1951.

(7) Difco Laboratories. *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures.* 9th ed. Difco Labs., Detroit. 1953.

(8) EASTERBROOKS, H. L., HELLER, P., LIEBERMAN, M., PLASTRIDGE, W. N., AND JUNGHERR, E. L. Fertility and Bacterial Content of Diluted Bull Semen Treated with Various Concentrations of Dihydrostreptomycin Sulfate. Am. J. Vet. Research, 12: 191-193. 1951.

(9) EASTERBROOKS, H. L., HELLER, P., PLASTRIDGE, W. M., AND JUNGHERR, E. L. Streptomycin and Streptomycin plus Penicillin for Increasing the Fertility of Diluted Bull Semen. North Am. Veterinarian, 32: 394-395. 1951.

(10) FOOTE, R. H., AND BRATTON, R. W. The Fertility of Bovine Semen in Extenders Containing Sulfanilamide, Penicillin, Streptomycin and Polymyxin. J. Dairy Sci., 33: 544-547. 1950.

(11) FOOTE, R. H., AND BRATTON, R. W. Motility of Bovine Spermatozoa and Control of Bacteria at 5 and 25° C. in Extenders Containing Sulfanilamide, Penicillin, Streptomycin, and Polymyxin. J. Dairy Sci., 33: 842-850. 1950.

(12) FOOTE, R. H., AND SALISBURY, G. W. The Effect of Sulfonamides upon the Livability of Spermatozoa and upon the Control of Bacteria in Diluted Bull Semen. J. Dairy Sci., 31: 769-778. 1948.

(13) JANEWAY, C. A. Method for Obtaining Rapid Bacterial Growth in Cultures from Patients under Treatment with Sulfonamides. J. Am. Med. Assoc., 116: 941-942. 1941.

(14) MCQUARRIE, E. B., AND LIEBMANN, A. J. Studies on Penicillinase. Arch. Biochem., 5: 307-315. 1944.

(15) MYERS, R. M., AND ALMQUIST, J. O. A Comparison of the Effects of Aureomycin, Penicillin, and Streptomycin upon Spermatozoan Livability and Control of Bacteria in Bovine Semen. J. Animal Sci., 10: 322-330. 1951.

(16) PLASTRIDGE, W. N., AND EASTERBROOKS, H. L. Effect of Antibiotics in Diluted Bull Semen on Vibrio Fetus. Am. J. Vet. Research, 13: 145-148. 1952.

(17) SALISBURY, G. W., AND KNODT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. J. Dairy Sci., 30: 361-369. 1947.

(18) SCHAUB, I. G., AND FOLEY, M. K. *Diagnostic Bacteriology.* 3rd ed. C. V. Mosby Co., St. Louis. 1947.

(19) STALLCUP, O. T., AND MCCARTNEY, H. K. Toxicity to Bull Spermatozoa of Terramycin Hydrochloride and Its Use as an Antibacterial Agent in Diluents. J. Dairy Sci., 34: 293-295. 1953.

PROTEOLYSIS BY *STREPTOCOCCUS LACTIS* GROWN IN MILK WITH AND WITHOUT CONTROLLED pH [1]

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Streptococcus lactis long has been recognized primarily as being saccharolytic. However, the results of various investigations have shown that many strains are capable of causing protein degradation in milk. Early studies concerning proteolysis by *S. lactis* in milk were reviewed by Hammer and Babel (5). In most of these studies proteolysis was determined by *(a)* Kjeldahl analysis of nitrogen soluble in acetic acid, trichloroacetic acid or phosphotungstic acid, *(b)* the determination of free amino acids by either the Van Slyke method or the formol titration, or *(c)* ammonia determinations. In most cases, milk inoculated with *S. lactis* was incubated for periods ranging from several days to months, with and without added calcium carbonate. Commonly, protein cleavage was greater with calcium carbonate present than without. Collins and Nelson (3) reported that *S. lactis* grown in skimmilk caused a considerable increase in the trichloroacetic acid-soluble nitrogen during the first 24 hours, with a gradual but smaller increase throughout the 15-day test period. Morgan and Nelson (9) showed marked increases of ten amino acids in tungstic and lactic acid filtrates of milk after incubation with *S. lactis* for 15 days at 21 ° C.

Proteolysis during the early phases of growth has received little or no attention, mainly because techniques commonly used did not show definite changes which could be associated with protein hydrolysis. The need to detect and determine smaller degrees of protein hydrolysis in milk has led to use of a modified test for tyrosine and tryptophan (6), which has proved to be extremely sensitive for determining the breakdown of milk proteins. In this method, tyrosine and tryptophan liberated from the protein are determined in a trichloroacetic acid filtrate of milk by measuring the blue color in a photometer after addition of Folin-Ciocalteau reagent (4). Recently a similar procedure was used successfully by Lembke *et al.* (8) in determining small changes caused in milk proteins by pepsin and trypsin. No reports have been found of any studies concerning proteolysis by *S. lactis* in milk with controlled pH.

EXPERIMENTAL METHODS

In this work proteolysis by *S. lactis* growing in skimmilk at 32° C. with and without controlled pH for periods up to 70 to 90 hours was studied. Nitrogen soluble and insoluble in trichloroacetic acid, total nitrogen, tyrosine and tryptophan in trichloroacetic acid filtrates, pH, and plate count were deter-

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mined. The three strains of *S. lactis* used were from a group of 13 pure cultures isolated from different samples of raw cream and were selected on the basis of their ability to produce the greatest increase in non-protein nitrogen when grown in milk. The streptococci were identified as *S. lactis* by the characteristics given by Sherman (11). The stock cultures were transferred weekly and were carried in sterile litmus milk. Cultures were transferred every day for three transfers prior to each trial. One-tenth of 1 per cent inoculum was used in all cases. Cultures were incubated at 32° C. Milk for the trials without controlled pH consisted of fresh skimmilk which was heated at 185° F. for 20 minutes to destroy as many undesirable organisms as possible without subjecting the milk constituents to changes that might occur during sterilization. Because of the absence of a rapid drop in pH in the cultures with controlled pH, milk for these samples and their controls was autoclaved for 12 minutes at 15 lb. pressure. Skimmilk was chosen because the absence of fat facilitated accurate sampling and analysis. In the trials with controlled pH, 200 ml. of sterile milk were pipetted into a sterile beaker covered by a double layer of sterile aluminum foil with holes (covered with sterile cotton) to fit the electrodes and stirrer of a Beckman Model K automatic titrator placed in a 32° C. incubator. After inoculation, the sample was kept at the desired pH by sodium hydroxide delivered from the automatic titrator. The accuracy of pH control was checked at regular intervals by determining the pH of the sample with a Beckman pH meter; deviations from the desired pH value were not more than $\pm$ 0.15 of a unit. Samples without controlled pH and without agitation were run at the same time in screw top bottles. Before inoculation, all samples were brought to the desired pH with sodium hydroxide or sulfuric acid. At certain intervals material for analysis was withdrawn from the samples and their controls with sterile pipettes. Changes in volume were recorded, and necessary corrections were made in the controls with sterile distilled water.

Nitrogen fractions insoluble in trichloroacetic acid were precipitated by the method developed for cheese serum by Lane and Hammer (7), except that in this work a 5-g. sample was used, whereas Lane and Hammer used 1 ml. of cheese serum. Portions of the coagulated cultures were blended for 5 minutes in a sterile Waring blender with a semi-micro attachment before final sampling. All nitrogen determinations were made in duplicate by the Kjeldahl procedure. Samples for bacterial counts were taken directly from the blender in case of coagulated cultures and from the sample beaker when the culture had not coagulated. Bacterial counts were made according to *Standard Methods for the Examination of Dairy Products* (1), using incubation at 32° C. A Beckman model G glass electrode pH meter was used for all pH determinations. Tyrosine and tryptophan were determined according to a method described by Hull (6), except that the trichloroacetic acid was added directly to the sample of the culture tested. The blue color which appears after adding Folin-Ciocalteau reagent to the protein-free filtrate was measured with a Klett-Summerson photoelectric colorimeter, using a 645 mμ wave-length filter. A standard tyrosine curve showing the per cent light transmission for various

tyrosine concentrations then was used to convert the sample reading to its tyrosine equivalent. It is recognized that these values represent not only tyrosine and tryptophan but also some of the smaller peptides containing these two amino acids in positions where they may react with the Folin-Ciocalteau reagent. Analyses of the samples without controlled pH were performed as outlined for the samples held at constant pH. Results were calculated on the basis of 5 ml. of original milk.

RESULTS

In early experiments, proteolysis by three strains of *S. lactis* growing in heated skimmilk without controlled pH was studied for periods up to 72 hours. The production of soluble nitrogen was fairly rapid during the first day, when the cell population reached a maximum and acid production was at the most rapid rate. Later, when the number of viable cells was declining and little or no acid was produced, the rate of production of soluble nitrogen also declined; however, there was a gradual increase in soluble nitrogen throughout the 72-hour test period, resulting in a conversion of 2.8 to 3.8 per cent of acid-insoluble nitrogen into the soluble form. In all cases the production of soluble nitrogen during the first 12-hour incubation period was negligible. When calcium carbonate was added to the milk (10 g. per 100 ml.), a final increase in soluble nitrogen approximately twice as great as without calcium carbonate was observed.

Representative data showing increases in the free tyrosine and tryptophan content of milk caused by *S. lactis* growing without pH control are presented in Table 1. In all samples definite increases in tyrosine and tryptophan were observed after a 4-hour incubation period. Approximately 35 (strain 18), 40 (strain 26), and 57 (strain 34) per cent of all tyrosine and tryptophan liberated during the full period of experimentation was freed during the first 12-hour incubation period, during which bacterial population and rate of acid production reached maxima. Although a gradual increase in tyrosine and tryptophan was found throughout the 90-hour incubation period, a progressive decline in rate of production was noticed after the number of viable cells and acid production reached or approached maximum levels. Addition of sterile lactic acid to heated skimmilk to give titratable acidities ranging from 0.2 to 1.0 per cent, followed by incubation at 32° C. for 24 hours, failed to bring about any increases in either soluble nitrogen or tyrosine and tryptophan, indicating that the protein breakdown was not due to the lactic acid produced by *S. lactis*.

Trials with controlled pH were run on strain 26 of *S. lactis*. In Figures 1 and 2 are presented representative results showing increases in soluble nitrogen and tyrosine and tryptophan of comparable samples with and without controlled pH. The milk used contained 3.36 mg. trichloroacetic acid-soluble nitrogen and 24.44 mg. trichloroacetic acid-insoluble nitrogen per 5 g. All cultures with controlled pH showed greater increases in soluble nitrogen, except for the sample held at pH 8.5, and also in tyrosine and tryptophan, except for the samples held at pH 8.0 and 8.5, than comparable samples

TABLE 1

Proteolysis by S. lactis grown in heated skimmilk

Age	Strain 18			Strain 26			Strain 34		
	pH	S.P.C.	Tyrosine[a]	pH	S.P.C.	Tyrosine[a]	pH	S.P.C.	Tyrosine[a]
(hr.)		*(millions/ml.)*	*(γ/5 ml. milk)*		*(millions/ml.)*	*(γ/5 ml. milk)*		*(millions/ml.)*	*(γ/5 ml. milk)*
0	6.60	5.8	0	6.65	31	0	6.60	4	0
4	6.30	640	30	6.25	510	30	5.95	75	50
8	4.75	2500	65	4.80	2200	90	4.30	1100	135
12	4.35	4000	95	4.40	3000	140	4.20	1600	190
24	4.20	2600	165	4.15	1800	225	4.10	640	265
36	4.10	2000	205	4.10	1600	280	4.10	560	295
48	4.10	320	230	4.05	175	315	4.10	56	325
72	4.10	0.9	250	4.10	0.6	340	4.05	0.2	330
90	4.10	....	270	4.10	....	350	4.10	....	335

[a] Increase in free tyrosine equivalent; unincubated milk contained equivalent of 225 γ per 5 ml.

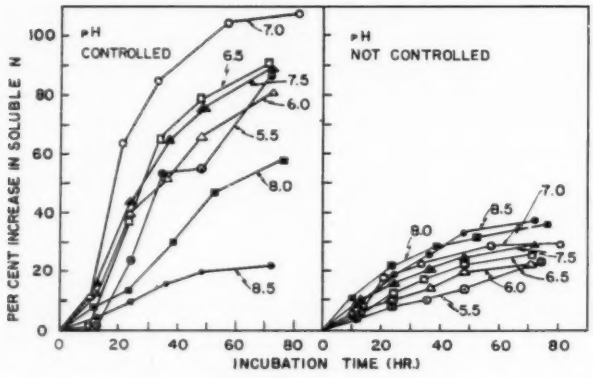

FIG. 1. Increases in soluble nitrogen per 5 g. sample in milk by *S. lactis* 26 at various pH levels with and without controlled pH (3.36 mg. acid-soluble nitrogen was present in 5 g. of the original milk)

without controlled pH; the maximum increase was at pH 7.0. More soluble nitrogen and free tyrosine and tryptophan were found in the samples held at pH 6.0 to 7.5 than at pH 5.5, 8.0, and 8.5. A somewhat delayed but marked increase in soluble nitrogen was observed at pH 5.5. Although the differences between the control samples at various pH levels seemed to be of minor importance, the amount of soluble nitrogen was greatest in the sample adjusted to pH 8.5 and decreased progressively as the initial reaction of the medium was dropped to pH 5.5. Presumably this was because the more alkaline samples

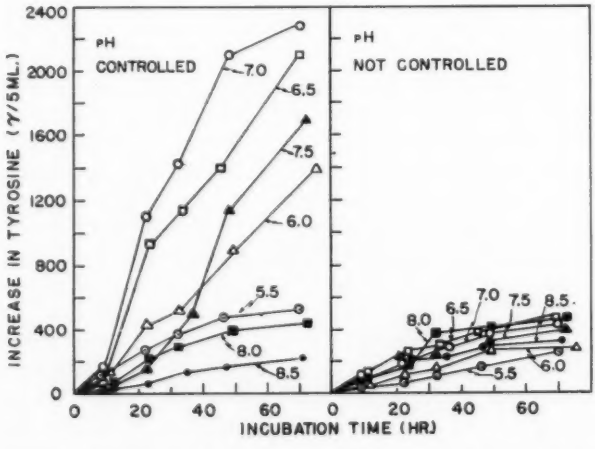

FIG. 2. Increases in tyrosine and tryptophan equivalent per 5 ml. sample in milk by *S. lactis* 26 at various pH levels with and without controlled pH (215 γ free tyrosine was present in 5 ml. of the original milk)

required a longer time to reach low pH levels. In Figure 3 are presented the bacterial counts of the samples with controlled pH and their comparable controls made at the time soluble nitrogen was determined. After the viable bacterial population had reached a maximum, little or no decline was observed in the samples with controlled pH; comparable controls showed a fairly rapid decline after approximately 24 hours incubation. Some differences in the number of viable bacteria at different pH levels were found. The number of viable cells in the culture maintained at pH 8.5 was throughout the experi-

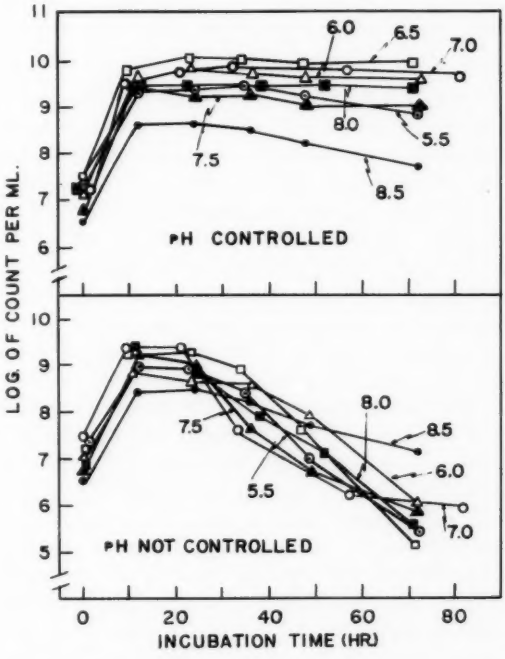

FIG. 3. Population changes of *S. lactis* 26 at various pH levels (Soluble N expts.).

mental period much lower than at the other pH values, probably because of the high pH. Similar results were obtained for the samples in which tyrosine and tryptophan were determined.

Since marked evidence of proteolysis was found in cultures of *S. lactis* after a short incubation period, the essentially cell-free culture medium was tested for proteolytic activity. The three strains of *S. lactis* were grown in *(a)* skimmilk; *(b)* simplified amino acid medium of Niven and Sherman (10); *(c)* vitamin-test casein medium, containing 20 g. vitamin-test casein (Nutritional Biochemicals), 6.5 g. glucose, 10 g. $K_2HPO_4 \cdot 3H_2O$, 10 g. yeast extract, and 1,000 ml. distilled water; *(d)* medium *C* in which vitamin-test casein was

replaced with an equal amount of technical casein; and *(c)* tryptone glucose meat extract broth.[2] *S. lactis* was grown in these culture media for periods ranging from 4 to 72 hours at 32° C., both without controlled pH and with reaction maintained at pH 6.5, followed by removal of the cells in a high speed centrifuge. Supernatant in quantities up to 5 ml. was added to 5 ml. skimmilk, followed by incubation at 32 and 37.5° C. for 12 to 36 hours. The skimmilk was adjusted to pH values ranging from 5.0 to 8.0. Toluene, and in later experiments merthiolate (1 mg. per 40 ml. substrate), was used to prevent bacterial growth during the incubation period. Blanks were prepared by adding the supernatant to the substrate after the trichloroacetic acid had been added. No increase in soluble nitrogen or tyrosine and tryptophan was found.

DISCUSSION

The patterns of the increases in soluble nitrogen and in tyrosine and tryptophan show that the former increases somewhat more slowly during the first 10 or 12 hours of incubation, using relatively heavy cell inocula. As the pH of the system begins to drop and the metabolic activity of the cells presumably begins to decline in the uncontrolled system, the rate of proteolysis, as determined by either procedure, declines. In the samples held at controlled pH levels from pH 6.0 to 7.5, both determination procedures indicate a continuing proteolysis. Obviously, the decline in proteolysis as the pH drops is not due to exhaustion of available substrate, for when the pH is maintained near neutrality the substrate continues to be attacked. The possibility exists that the substratum would not be as available for enzyme action at the lower pH levels. Whether or not the pH is maintained, there is little probability that the cells are destroyed by autolysis; although the numbers of viable cells decline more rapidly in the runs with uncontrolled pH, this need not lead to rapid cell autolysis. The greater proteolytic activity at pH levels of 6.0 to 7.5 might be due to greater enzyme production, activity, or stability at these levels of reaction. The inability to detect proteolytic activity of either type in culture media of various types after the cells had been removed by centrifugation prevented study of the characteristics of the enzyme system in this manner. If an extra-cellular enzyme system is produced under these experimental conditions it obviously either is not stable or is inactive under the conditions used for determination. The responsible enzyme systems also might be closely associated with the cell. A subsequent paper will cover studies on this phase of the investigation.

The earlier appearance of tyrosine and tryptophan than of increased soluble nitrogen would indicate either that two enzyme systems are involved or that the freeing of compounds giving tyrosine and tryptophan reactions with the Folin-Ciocalteau reagent is an early phase of the proteolytic activity. The greater rates of increase in soluble nitrogen at pH 5.5 and, to some degree, at pH 8.0 than of increase in tyrosine and tryptophan at these pH levels in-

[2] Difco Laboratories, Detroit, Michigan.

dicate the possibility of the two systems. By calculation from average values (2), from 2.0 to 2.7 per cent of the total tyrosine and tryptophan was liberated in 72 hours in cultures without controlled pH, whereas the same figures relative to soluble nitrogen were 2.8 to 3.8 per cent. In the sample maintained at pH 7.0 for 70 hours, 18.5 per cent of the tyrosine and tryptophan present in milk proteins was liberated, whereas the same figure for soluble nitrogen was 14.8 per cent. However, the relative closeness of these figures probably is fortuitous. Study of enzyme systems freed from the cells will be necessary to determine definitely whether more than one enzyme is involved.

SUMMARY

S. lactis organisms caused a rapid increase in both soluble nitrogen and tyrosine and tryptophan during the first 24 hours, followed by a smaller but gradual increase during the rest of the experimental period (72 to 90 hours). Marked increases in tyrosine and tryptophan were found when the production of soluble nitrogen as determined by the Kjeldahl procedure still was negligible. Considerably more soluble nitrogen and tyrosine and tryptophan were produced when the reaction was controlled at pH 6.0 to 7.5 thar in comparable samples without controlled pH. The cell-free culture medium did not show proteolytic activity.

REFERENCES

(1) American Public Health Association. *Standard Methods for the Examination of Dairy Products.* 9th ed. American Public Health Assoc., New York. 1948.

(2) Block, R. J., and Bolling, D. *The Amino Acid Composition of Proteins and Foods.* 2nd ed. C. C. Thomas, Springfield, Ill. 1950.

(3) Collins, E. B., and Nelson, F. E. The Effect of *Streptococcus lactis* and Coliform Organisms on the Soluble Nitrogen of Milk. J. Dairy Sci., **32**: 652-658. 1949.

(4) Folin, O., and Ciocalteau, V. On Tyrosine and Tryptophan Determinations in Proteins. J. Biol. Chem., **73**: 627-650. 1927.

(5) Hammer, B. W., and Babel, F. J. Bacteriology of Butter Cultures: A Review. J. Dairy Sci., **26**: 83-168. 1943.

(6) Hull, M. E. Studies on Milk Proteins. II. Colorimetric Determination of the Partial Hydrolysis of the Proteins in Milk. J. Dairy Sci., **30**: 881-884. 1947.

(7) Lane, C. B., and Hammer, B. W. Bacteriology of Cheese. I. Effect of Pasteurizing the Milk on the Nitrogenous Decomposition of Cheddar Cheese. Iowa Agr. Expt. Sta. Research Bull. 183. 1935.

(8) Lembke, A., Kaufmann, W., and Schmidt, H. Enzymatische Studien an Milchproteinen. Milchwissenschaft, **8**: 10-16. 1953.

(9) Morgan, M. E., and Nelson, F. E. The Distribution of Certain Amino Acids in Soluble Fractions of Milk Cultures of *Streptococcus lactis.* J. Dairy Sci., **34**: 446-456. 1951.

(10) Niven, C. F., Jr., and Sherman, J. M. Nutrition of the Enterococci. J. Bacteriol., **47**: 333-342. 1944.

(11) Sherman, J. M. The Streptococci. Bacteriol. Revs., **1**: 1-87. 1937.

STUDIES OF HERD MANAGEMENT RECORDS
I. SERVICES REQUIRED FOR CONCEPTION FOR FIRST TO TENTH CALVING

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Successful herd operation depends not only upon the production of large quantities of milk and butterfat by the cows but also upon their ability to drop calves with regularity. A calf a year is the suggested ideal. When this is attained, a cow during her useful life in the herd usually will have a larger total production than when calving occurs at longer intervals. Another ideal, one service per conception, has never been attained over a period of years for a herd of any considerable size. What influence successive calvings may have upon the conception rate never has been fully explored. The percentage of conceptions that occur at various services for successive calvings also has been incompletely reported. This study is an attempt to present data bearing on these problems.

REVIEW OF LITERATURE

The influence of abortion upon services for conception has been discussed by White *et al.* (12), who found that negative reacting cows required fewer services than positive abortion reactors. Eckles (2) reported that when the average abortion rate was 14.6%, 2.52 services were needed for each conception. Morgan and Davis (9) at an earlier date indicated that 2.21 services were required per conception when the abortions averaged 12.5%. They further reported that virgin heifers with 2.52 services per conception required more services than were needed for females at any subsequent calving up to the tenth. That more services are needed for conception by first calf heifers than for older cows was shown by the work of Gowen and Dove (4); Hilder *et al.* (5); Foot and Ridler (3); Kinsman (6), and Bowling *et al.* (1). Knoop and Hayden (7) found age to be no factor.

The percentage of conception at three services for the first and subsequent calvings is another measure of breeding efficiency. The percentages of cows that conceived at three services for the first and for all calvings as reported by various investigators is as follows: Gowen and Dove (4), first calving 60.0, all calvings 89.9; Hilder *et al.* (5), first calving 60.8, all calvings 69.6; Stans-

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bury (11), first calving 60.0, all calvings 86.0; Foot and Ridler (3), first calving 53.6, all calvings 79.6; Olds and Seath (10), first calving 68.5, all calvings 97.9; Kinsman (6), first calving 52.2, all calvings 91.2; and Miller and Graves (8), first calving 67.4, all calvings 73.9.

SOURCE OF DATA

The University of Nebraska has maintained a herd of purebred Holstein-Friesians since 1896 under a relatively uniform management program. The average yearly production of butterfat has been increased about 200 lb. per cow. Complete records have been kept with reference to abortion, services, and calving. The data here presented were obtained during the period 1904 through 1948 and involve 437 fertile cows, as determined by subsequent calvings. There were 980 calvings, of which 100 were abortions. The cows were mated naturally until 1936, after which artificial insemination was practiced. Each cow was tested for production at each lactation; and at various periods, four-, three-, and two-time milking was practiced. Of the cows included in the study, 11 produced 9 calves each; 8 produced 10 each; 3 produced 11 each, and 1 produced 14. Because of the small numbers involved, tenth and subsequent calvings were grouped together.

RESULTS AND DISCUSSION

For the 980 calvings involved 2,467 services were required, or an average of 2.52 services per conception. By calvings the average services needed for each conception were as follows: 1st, 2.93; 2nd, 2.40; 3rd, 2.22; 4th, 1.89; 5th, 2.48; 6th, 2.08; 7th, 2.38; 8th, 2.68; 9th, 3.00; 10th and subsequent calvings, 4.81.

Table 1 presents in percentages the results of the study. It will be noted that the percentage of conceptions at first service starts with 37.5 and reaches a high of 59.1 at the fifth calving, after which there is a somewhat irregular decline. The average for all calvings was 44.6%. Considering the first three services, the conception rate increased from 68.9% for the first calvings to 90.6% at the fourth, after which there was an irregular decline to a low of 20.0% for the tenth and subsequent calvings. Considering all calvings, 78.3% of the cows conceived at three services. For all calvings, the first five services yielded a conception percentage of 90.8.

Although the data are not presented because of space limitations, a tabulation similar to Table 1 was made, which, instead of considering all calvings, was concerned only with those that were normal and were preceded by normal calvings. This reduced the number of cows to 387, the number of conceptions to 886, and the number of services for conception to 2,257. In this tabulation the average number of services for each conception was 2.55 as compared with 2.52 for all calvings. Other comparisons were made as follows: conceptions at first service, all calvings, 43.7% (44.6 in Table 1); percentage of conception at three services, all calvings, 78.3 (78.3 in Table 1), and percentage of conception at five services, all calvings, 90.6 (90.8 in Table 1). It is believed

TABLE 1

Comparison of conception at various calvings, first through tenth by services

Service	1st calving	2nd calving	3rd calving	4th calving	5th calving	6th calving	7th calving	8th calving	9th calving	10th and later calvings	All calvings	
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(No.)*	*(%)*
1st	37.5	43.2	51.0	54.7	59.1	49.0	35.3	42.1	36.4	18.7	437	44.6
2nd	17.4	22.7	19.6	23.6	14.1	21.6	29.4	15.8	18.2	18.7	196	20.0
3rd	14.0	13.6	11.8	12.3	14.1	17.6	20.6	10.5	9.1	12.5	134	13.7
Total 1st 3 services	68.9	79.5	82.3	90.6	87.3	88.2	85.3	68.4	63.7	20.0	767	78.3
4th	9.0	7.7	7.2	3.8	1.4	7.8	8.8	10.5	9.1	6.2	71	7.2
5th	8.0	4.5	4.6	3.8	1.4	.....	2.9	10.5	18.2	6.2	52	5.3
Total 1st 5 services	85.9	91.8	94.1	98.1	90.1	96.1	97.1	89.5	90.9	62.5	890	90.8
6th	6.7	4.1	2.0	0.9	1.4	2.0	.....	5.3	.....	12.5	38	3.9
7th	3.3	1.8	4.6	.....	1.4	.....	.....	5.3	.....	12.5	22	2.2
8th	1.3	1.4	.....	.....	4.2	.....	.....	.....	9.1	.....	11	1.1
9th	1.3	0.4	0.6	.....	.....	.....	.....	.....	.....	.....	6	0.6
10th	1.3	0.4	0.6	0.9	2.8	2.0	2.9	.....	.....	12.5	13	1.3
Total 10 services	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	980	100.0

that the elimination of the abortions had no appreciable effect upon the services required for conception or the percentages that conceived at one, three, or five services. All the figures in both tabulations were extremely close. Since a relatively small percentage of cows conceived after the fifth service, it is questionable if any but very valuable breeding cows should be retained if conception has not taken place by the fifth service.

Statistical tests for significance were applied, using the Chi-square method of Snedecor with the following results: When a comparison of the conception percentage for the first service, for three services, and for five services was made for the first calvings with the sum of the fourth, fifth, sixth, and seventh calvings, each comparison yielded a Chi-square value that was significantly lower — <0.01 for the first calving. When all services for the first calvings were compared with the sum of the services for the fourth, fifth, sixth, and seventh calvings, the Chi-square value was significantly higher — <0.01 for the first calving.

SUMMARY

Tabulation of 980 calvings of 437 Holstein-Friesian cows in the University of Nebraska herd in a 44-year period, during which the abortion rate was 10.2%, showed that:

1. First calf heifers required the largest number of services for conception. This number decreased up to the fourth calving.
2. First calf heifers have a lower conception rate than do mature cows when measured by percentage of conception at first service, at first three services, and at first five services.
3. There is a gradual increase in the conception percentage for each calving for the first through the fourth, as measured by first service, first three services, and first five services.
4. From the fourth through the seventh calvings, the conception percentage remained relatively the same.
5. There was a decline in fertility after the eighth calving.
6. Comparison of these data with those based on normal calvings after normal calvings showed no difference; hence, abortion was judged to be an unimportant factor as affecting the factors studied.

ACKNOWLEDGMENT

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REFERENCES

(1) Bowling, G. A., Putnam, D. N., and Ross, R. H. Age as a Factor Influencing Breeding Efficiency in a Dairy Herd. J. Dairy Sci., 23: 1171-1176. 1940.

(2) Eckles, C. H. A Study of Breeding Records of Dairy Herds. Minn. Agr. Expt. Sta. Bull. 258. 1929.

(3) Foot, A. S., and Ridler, B. Dairy Herd Fertility. Empire J. Exptl. Agr., 17: 229-237. 1949.

(4) Gowen, J. W., and Dove, W. F. Fertility in Dairy Cattle. Me. Agr. Expt. Sta. Bull. 360. 1931.

(5) Hilder, R. A., Fohrman, M. H., and Graves, R. R., Relationship of Various Factors to Breeding Efficiency of Dairy Animals and the Sex Ratio of the Offspring. J. Dairy Sci., 27: 981-992. 1944.

(6) Kinsman, D. M. The Relation Between Age and Rate of Conception in Dairy Cattle Bred Artificially. Guern. Breed. J., 84: 2: 166-167. 1952.

(7) Knoop, C. S., and Hayden, C. C. Study of the Length of Gestation and Service Record. Ohio Agr. Expt. Sta. Bimonthly Bull. 166. 1934.

(8) Miller, F. W., and Graves, R. R. Reproduction and Health Records of the Beltsville Herd of the Bureau of Dairy Industry. U.S.D.A. Tech. Bull. 321. 1932.

(9) Morgan, R. F., and Davis, H. P. Influence of Age of Dairy Cattle and Season of the Year on the Sex Ratio of Calves and the Number of Services Required for Conception. Nebr. Agr. Expt. Sta. Research Bull. 104. 1938.

(10) Olds, D., and Seath, D. M. Predictability of Breeding Efficiency in Dairy Cattle. J. Dairy Sci., 33: 721-724. 1950.

(11) Stansbury, J. L. Reproduction of Dairy Cattle. Ohio Agr. Expt. Sta. Bull. 294. 1948.

(12) White, G. C., Johnson, R. E., Rettger, L. L., and McAlpine, G. Some Economic Phases of Bacterium Abortus Infections and Other Observations in Dairy Cattle. Conn. (Storrs) Agr. Expt. Sta. Bull. 135. 1925.

AMINO ACID METABOLISM OF BOVINE RUMEN BACTERIA[1]

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Several workers have recognized the importance of nitrogen metabolism involving microorganisms of the rumen. However, studies on the degradation of protein, and its derivatives, by rumen bacteria are few in number. In vivo studies by McDonald (13) showed that, in addition to naturally occurring plant protein, other purified proteins of animal origin were readily attacked by rumen microorganisms with the production of ammonia. El-Shazly (9) has demonstrated the formation of ammonia and fatty acids from mixed amino acids when suspensions of rumen bacteria were employed. Nitrogen metabolism of rumen bacteria tends to be obscured by the predominant role accorded cellulose and starch degradation reactions. However, interest is evinced in microbial reactions involving nitrogenous compounds when nonprotein nitrogen substitutions, viz., urea, are studied. Various bacteriological aspects of the nitrogen metabolism of the rumen have been reviewed by Doetsch and Robinson (4).

In this paper are presented results of studies on the degradation of individual amino acids by mixed suspensions of bovine rumen bacteria. Further, the effect of carbohydrates and other factors on the metabolism of amino acids is examined. The authors are aware of the pitfalls involved in transferring data derived from work of this sort in toto to constructions of reactions involved in the rumen. It is reasonable to assume, however, that some useful working approximations might be made. Limitations of this method have been pointed out by Doetsch *et al.* (5) in a study of carbohydrate metabolism, using these same techniques.

EXPERIMENTAL

Collection and processing of rumen samples and preparation of the mixed suspensions were carried out as previously described (5). Manometric techniques also were the same. Dissimilation tests were modified as follows: 125-ml. Erlenmeyer flasks contained 150 micromoles of substrate in 12 ml. of previously boiled and cooled 0.066 *M* phosphate buffer (pH 6.9) with 0.02% (w/v) $Na_2S \cdot 9H_2O$. Four ml. of cell suspension were added to each flask. Incubation was carried out at 39° C. for 72 hours. After this period, 4 ml. of 10 *N* H_2SO_4 were added to each flask to stop the reaction.

Determinations of ammonia, keto acids, and lactic acid were performed colorimetrically by methods of Conway (3), Friedmann and Haugen (10), and Barker and Summerson (1), respectively. Fatty acids were determined

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chromatographically by the method of Elsden (7) except that the "alphamine Red R" indicator of Liddel and Rydon (11) was used instead of brom-cresol green. Recovery of 95% of known volatile fatty acids, both single and mixed, was obtained in control determinations.

Colorimetric determinations were repeated several times, and results showed a variation within a range of 5%. The data presented here either are typical or represent the general trend.

The L forms of the following amino acids were tested: glycine, alanine, β-alanine, valine, leucine, isoleucine, serine, threonine, cysteine, cystine, methionine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophane, proline, hydroxyproline, histidine, arginine, lysine, and ornithine.

RESULTS

Decarboxylation and deamination occurred with only aspartic acid, glutamic acid, serine, arginine, cysteine, and cystine. Further analytical data derived from experiments with these amino acids are presented in Table 1. Analysis

TABLE 1

Amino acid decomposition by mixed suspensions of bovine rumen bacteria as measured manometrically

Substrate	μL Gas	μL CO_2	μM CO_2	μM NH_3
Aspartate	112.0[a]	112.0	5.0	4.85
Glutamate	24.7	24.7	1.1	1.17
Serine	17.9	17.9	0.8	1.2
Arginine	10.1	10.1	0.45	1.77
Cysteine	61.0	33.5	1.5	1.46
Cystine	50.0	23.7	1.06	1.2
Urea	110.0	110.0	4.9	10.0

[a] Figures represent total values obtained after 2 hr. incubation at 39° C.

of these results show that both aspartic acid and urea were attacked at a rate approximately five times greater than for the remaining five amino acids. Equal amounts of both carbon dioxide and ammonia were produced in each instance from all six amino acids. In addition, a considerable amount of gas other than carbon dioxide was evolved from cysteine and cystine.

Results of the large-scale dissimilation tests are presented in Table 2.

TABLE 2

Amino acid decomposition by mixed suspensions of bovine rumen bacteria as measured by the large-scale dissimilation method

Substrate	Theoretical NH_3 yield	μM NH_3 prod.	μM keto acids	μM lactic acid	H_2S prod.	μM of Fatty Acids			
						Total	Acetic	Propionic	Butyric
Aspartate	150	145[a]	0	0	—	124.3	50.1	64.0	9.6
Glutamate	150	156	0	0	—	133.0	101.8	9.9	21.7
Serine	150	147	0	0	—	141.2	111.0	11.7	18.2
Arginine	600	277	0	0	—	98.0	53.2	14.2	30.2
Cysteine	150	133	0	0	+	127.0	89.3	23.5	14.2
Cystine	300	165	0	0	+	145.0	107.3	14.7	22.6

[a] Figures represent total values obtained with 150 micromoles of each substrate after 72 hr. incubation at 39° C.

Aspartic acid, glutamic acid, serine, and cysteine were nearly completely dissimilated in 72 hours. Arginine and cystine were incompletely utilized. Lactic and keto acids were not found as end-products from any of these substrates. The addition of 10% lead acetate solution to 0.1 ml. of reaction mixture gave qualitative tests for hydrogen sulfide (controls were negative). The formation of this gas probably accounts for the volume of gas other than carbon dioxide evolved from cysteine and cystine.

In each case acetic, propionic, and butyric acids were produced from all six amino acids. Glutamic acid, serine, arginine, cysteine, and cystine were dissimilated mainly to acetic acid, whereas propionic acid was the principal fatty acid derived from aspartic acid.

It was considered desirable to know the optimum substrate concentration at which complete dissimilation would occur during the test period in manometric studies. To accomplish this, a constant amount of mixed suspension was incubated with three different concentrations of aspartic acid. Figure 1

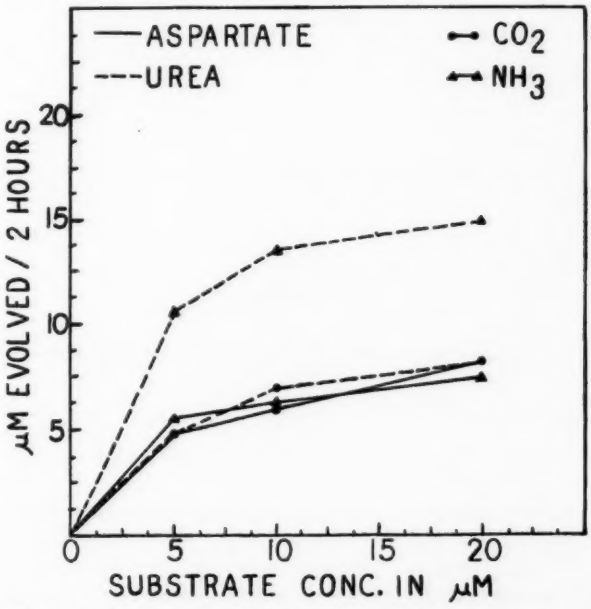

FIG. 1. The effect of substrate concentration on the dissimilation of aspartic acid and urea.

shows that 5 micromoles was the maximum amount that could be completely dissimilated in 2 hours. A simultaneous experiment with urea resulted in a similar value. While this experiment gives no information relative to the concentrations required for maximal rate of dissimilation it does indicate the limiting quantity at which total conversion of substrate during the test period may be achieved.

Measurement of total carbon dioxide evolution and ammonia produced from aspartic acid at various pH levels resulted in the curves shown in Figure 2. Maximum production of ammonia and carbon dioxide occurred around pH 6.9.

In Figure 3 data are presented showing the effect of the addition of 2.5 micromoles of maltose on the dissimilation of 5 micromoles of aspartic acid. By use of replicate flasks the reactions were stopped at various intervals during an 80 minute incubation period. It was observed that significant en-

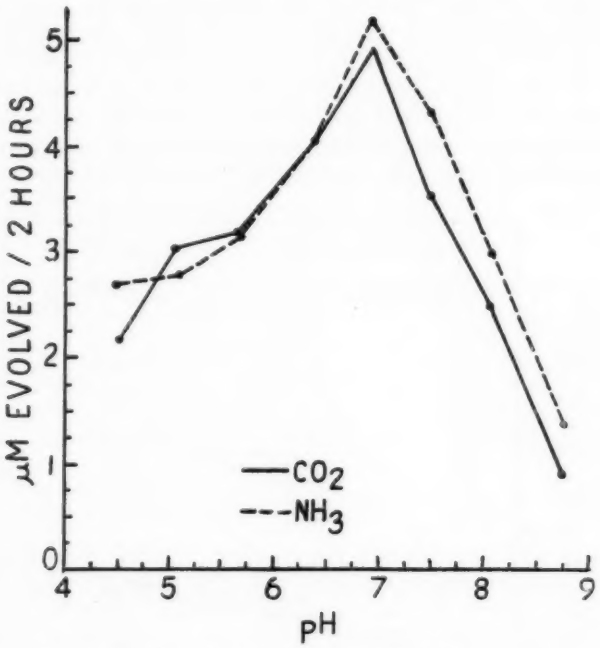

FIG. 2. The effect of pH on the dissimilation of aspartate.

hancement of both decarboxylation and deamination occurred in the presence of maltose. Similar, but less vigorous, stimulation was obtained with cellobiose and glucose. It can be seen that stimulation apparently ceased at the point at which the carbohydrate became exhausted (about 40 minutes). Additional manometric experiments utilizing the other five amino acids were performed, but since they are attacked at a less vigorous rate, all determinations were unsuccessful. In contradistinction to the failure of manometric studies, successful stimulation of deamination of these five amino acids (cysteine, cystine, arginine, serine, glutamic acid) by maltose occurred when large-scale dissimilation experiments were performed. In this case 50 micromoles of each amino acid were used with 50 micromoles of maltose. Ammonia determinations after 4 and 24 hours resulted in the data given in Table 3.

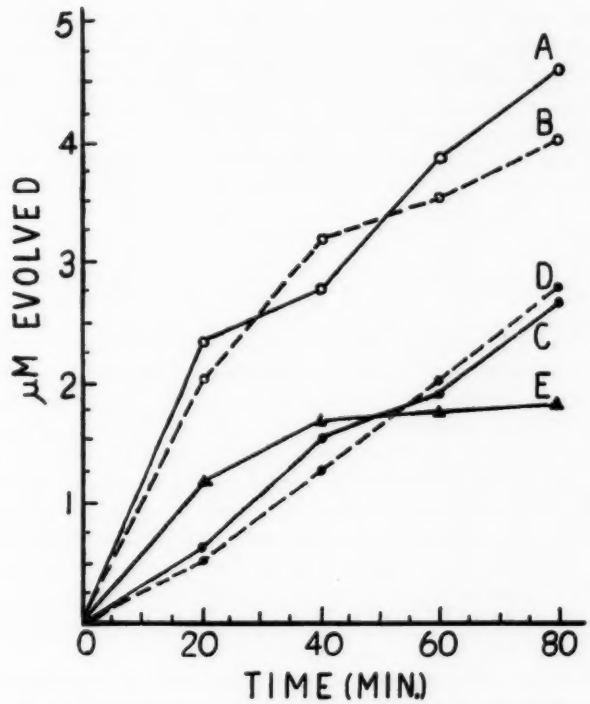

FIG. 3. The effect of maltose on the dissimilation of aspartate.
A. CO_2[a] evolved from aspartate in the presence of maltose.
B. NH_3 evolved from aspartate in the presence of maltose.
C. CO_2 evolved from aspartate alone.
D. NH_3 evolved from aspartate alone.
E. gas evolved from maltose alone.
[a] These values *do not* include the carbon dioxide evolved from maltose.

DISCUSSION

Although dissimilation reactions were observed to occur with only six of the amino acids tested, the experimental evidence by no means excludes the possibility of similar reactions occurring with the other 16 amino acids. If reactions involving these amino acids do occur, it is likely that they are of a magnitude too low to be detected by the methods used. Also, a number of these amino acids are known to be synthesized by rumen microorganisms. Evidence presented by Loosli *et al.* (12), Duncan *et al.* (6), and Black *et al.* (2) has demonstrated definitely that rumen organisms may synthesize essential amino acids. Consequently, reactions involving these amino acids might be entirely assimilatory in nature.

The amino acids ought to function prominently in the chemical economy of the rumen. Their dissimilation aids in supplying ammonia required for bac-

TABLE 3

Ammonia production from amino acids in the presence of maltose as determined with the large-scale dissimilation method

Substrate	μM ammonia produced			
	4 hr.		24 hr.	
	With maltose	Without maltose	With maltose	Without maltose
Glutamate	14.8	....	26	20
Serine	12.3	8.2	24	16
Arginine	16.4	5.8	48	34
Cysteine	12.3	5.8	27	21
Cystine	10.6	7.3	83	77

terial (McDonald, 13) and, possibly, host protein synthesis. In addition, the fatty acids, residues identical to the degradation products of carbohydrate metabolism in the rumen, can be absorbed as readily by the host animal. The accumulation of equal amounts of ammonia and fatty acids as end products in protein dissimilation has been shown previously by El-Shazly (8).

Since amino acid deaminases and decarboxylases have been reported to have widely differing pH optima for formation and activity, it is interesting to note that maximum carbon dioxide and ammonia production occurred at approximately pH 6.9. This might indicate the presence of a decarboxylase specific for the deamination product of aspartic acid rather than the amino acid itself. This reaction is now being clarified in this laboratory at the present time.

The observation that aspartic acid is most actively attacked may be significant for various reasons, among them the possible prominence of aspartic acid as an intermediate in the degradation of plant protein which would necessitate the presence of a proportionately greater amount of the specific enzyme.

The effect of carbohydrates on deamination probably is concerned with the activity of the preformed enzyme, since the organisms are assumed to be in a resting stage. This observation is in agreement with those of other workers who have recently demonstrated a similar effect using microorganisms not of rumen origin.

The significance of these studies lies in the fact that once basic mechanisms are found to occur, organisms specifically capable of carrying them out may be studied. Specific media can be devised for their identification and the task of following them through different diets, ages and conditions is simplified. In addition, our knowledge of the role played by bacteria is enlarged and will enable us ultimately to control the rumen flora and also detect abnormal conditions therein and perhaps correct for them by proper administration of specific compounds.

SUMMARY

Of 22 amino acids tested, aspartic acid, glutamic acid, serine, arginine, cysteine, and cystine were found to be attacked by suspensions of mixed rumen microorganisms. The principal degradation products detected were carbon

dioxide, ammonia, acetic acid, propionic acid, and butyric acid. The optimum pH for amino acid dissimilation appeared to be 6.9. A marked enhancement of amino acid dissimilation by a fermentable carbohydrate was demonstrated. The presence of maltose, cellobiose, and glucose significantly increased the production of ammonia and carbon dioxide from aspartic acid. The production of ammonia from glutamic acid, serine, arginine, cysteine, and cystine was similarly affected by the presence of maltose.

REFERENCES

(1) Barker, S. B., and Summerson, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. J. Biol. Chem., **138**: 536-554. 1941.

(2) Black, A., Kleiber, M., and Smith, A. H. Carbonate and Fatty Acids as Precursors of Amino Acids in Casein. J. Biol. Chem., **197**: 365-370. 1952.

(3) Conway, E. J. *Microdiffusion Analysis and Volumetric Error.* 3rd ed., Crosley Lockwood, London. 1950.

(4) Doetsch, R. N., and Robinson, R. Q. The Bacteriology of the Bovine Rumen: A Review. J. Dairy Sci., **36**: 115-142. 1953.

(5) Doetsch, R. N., Robinson, R. Q., Brown, R. E., and Shaw, J. C. Catabolic Reactions of Mixed Suspensions of Bovine Rumen Bacteria. J. Dairy Sci., **36**: 825-831. 1953.

(6) Duncan, C. W., Agrawala, I. P., Huffman, C. F., and Luecke, R. W. A Quantitative Study of Rumen Synthesis in the Bovine on Natural and Purified Diets. II. Amino Acid Content of Mixed Rumen Proteins. J. Nutrition, **49**: 41-50. 1953.

(7) Elsden, S. R. The Application of the Silica Gel Partition Chromatogram to the Estimation of Volatile Fatty Acids. Biochem. J., **40**: 252-256. 1946.

(8) El-Shazly, K. Degradation of Protein in the Rumen of the Sheep. 1. Some Volatile Fatty Acids, Including Branched Chained Isomers, Found *in vivo*. Biochem. J., **51**: 640-647. 1952.

(9) El-Shazly, K. Degradation of Protein in the Rumen of the Sheep. 2. The Action of Rumen Microorganisms on Amino Acids. Biochem. J., **51**: 647-653. 1952.

(10) Friedmann, T. E., and Haugen, G. E. Pyruvic Acid. 2. The Determination of Keto Acids in Blood and Urine. J. Biol. Chem., **147**: 415-442. 1943.

(11) Liddel, H. F., and Rydon, H. N. A New Indicator for Use in Partition Chromatography: 3, 6-disulpho-β-napthalene-azo-N-phenyl-α-napthylamine. Biochem. J., **38**: 68-70. 1944.

(12) Loosli, J. K., Williams, H. H., Thomas, W. E., Ferris, F. H., and Maynard, L. A. Synthesis of Amino Acids in the Rumen. Science, **110**: 144-145. 1949.

(13) McDonald, I. W. The Role of Ammonia in Ruminal Digestion of Protein. Biochem. J., **51**: 86-90. 1952.

PROTEOLYTIC ENZYMES FROM *BACTERIUM LINENS*

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The role of *Bacterium linens* in the ripening of limburger cheese is thought to involve the secretion of proteolytic enzymes which diffuse into the curd. The action of these enzymes upon the curd contributes to the characteristic body and flavor development. Although the relationship between *B. linens* and other organisms in the surface smear has been elucidated to a large extent (6, 8, 13), relatively few investigations have been concerned with the proteolytic enzymes secreted by *B. linens*. The partial hydrolysis of protein in milk cultures, after extended incubation, has been observed (1, 17) and enzymes from autolyzed cells and growth medium which attack casein, gelatin, and peptone have been studied in some detail (20).

The purpose of the experiments reported here was to elucidate the nature of the proteolytic enzymes produced by *B. linens* and to establish conditions for enzyme production in liquid cultures. It was found that an extracellular proteinase was produced which could be recovered from the growth medium by ammonium sulfate precipitation. Such preparations, which contained little peptidase and no polypeptidase activity, attacked only casein with the formation of a trichloroacetic acid-soluble polypeptide. Little proteinase activity was found in the cells.

MATERIALS AND METHODS

Bacteriological. Inocula of *Bacterium linens*, 450, were grown in a liquid medium composed of 1.0% yeast extract, 1.0% tryptone, 0.5% dipotassium phosphate (23), and 5.0 ml. of salts B (15) per liter. For enzyme production, 500 ml. of the above medium in 2-l. Fernbach flasks were inoculated with approximately 3.0 ml. of a 48-hour broth culture and incubated at 25°C. for 2 days on a rotary shaker.

The cells from a liter of medium were harvested and washed four times with 0.85% NaCl by centrifugation at 4° C., suspended in 75 ml. of saline with carborundum (20 mg. per milliliter), and disintegrated by sonic oscillation for 45 minutes in a Raytheon 10-KC, 200-watt oscillator. The clear extract was separated from the carborundum and cell debris by centrifugation and then dialyzed in a Visking casing against running tap water at 4° C. for 20 hours.

The extracellular proteinases were obtained from the medium after removal of cells. Concentration of the proteolytic activity was accomplished by ammonium sulfate precipitation, dissolution of the protein in 1/20 the original volume

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of distilled water, and dialysis as above. Such preparations were stable for at least 6 weeks when stored at —15° C.

Chemical. Approximately 25 g. of General Biochemical (GBI) casein, the principal substrate, were suspended in a small amount of water, 200 ml. of *N* NaOH added to dissolve the casein, and the pH adjusted to 7.2 with *N* HCl. After filtering to remove denatured material and autoclaving at 121° C. for 8 minutes, this preparation usually contained 7.0 to 7.5% protein. Protein concentration was adjusted to 6.6% with sterile distilled water before use. Crystalline bovine serum albumin, bovine γ-globulins, β-lactoglobulin, all purchased from Armour and Company, bovine seminal proteins (supplied by Dr. B. L. Larson), GBI lactalbumin, and α- and β-casein fractions (11), supplied by Dr. T. L. McMeekin, were used as substrates. A polypeptide, prepared by the action of the extracellular enzymes of *B. linens* on casein, also was used as a substrate. Polypeptide formation was stopped after 6 hours by the addition of trichloroacetic acid (TCA) and the mixture filtered. The TCA was removed by continuous extraction with ether for 24 hours and the volume concentrated in vacuo. The concentration of polypeptide was 22.4 mg. per milliliter. Glutathione, glycylglycylglycine, alanylphenylalanine, glycyltyrosine, and leucyltyrosine, all obtained from Nutritional Biochemicals, Inc., were substrates in peptidase studies.

Protein determinations were made by a modification of the biuret method of Robinson and Hogden (14). The method was useful in the range of 0.6 mg. to 25.0 mg. of casein, and replicate trials had a coefficient of variation (c.v.)[2] of 3%. The more rapid biuret method of Johnson and Swanson (7) (range of 1.0 mg. to 18.0 mg. of protein, c.v. 5%) also was employed. In addition, protein was determined by a modification of the spectrophotometric method of Snoke and Neurath (16) (range of 0.20 mg. to 4.0 mg. of protein, c.v. 1.5%).

The TCA-soluble polypeptides were estimated by the biuret method (14), using casein as a standard. Aromatic amino acids were measured spectrophotometrically at 280 mμ. Some interference by TCA was observed in these measurements. Amino acids and biuret-negative peptides were determined by the ninhydrin method of Moore and Stein (12), using alanine as a standard (range of 1.5 to 40.0 γ of alanine, c.v. 12%). Polypeptides in the TCA-soluble fraction were determined by this method after hydrolysis with 4 *N* HCl for 5 hours at 121° C. in tubes covered with inverted beakers. The hydrolysis of polypeptides and peptides was observed qualitatively by the paper chromatographic procedures of Consden, Gordon, and Martin (3).

Enzyme assay. Proteinase activity was assayed by incubating 6.0 ml. of culture filtrate or equivalent amounts of ammonium sulfate concentrated preparation with 3.0 ml. of 6.6% casein and 3.0 ml. of borate-phosphate buffer, pH 8.0 to 8.1, (pH of reaction mixture 7.2). After incubation at 38° C., aliquots of the reaction mixture were added to TCA. The TCA-insoluble fraction was analyzed

[2] $\text{c.v.} = \frac{\text{Standard deviation}}{\text{mean}} \times 100$

for protein and the TCA-soluble fraction, referred to as "TCA filtrate," was analyzed for amino acids and peptides.

RESULTS

Organism. Four strains of *Bacterium linens*, 450, 456, 462, and 476 (ATCC 9175), from the department collection, were tested for proteolytic activity by measuring the disappearance of casein in 1½ hours. The growth medium from 2-day cultures of strain 450 contained the most enzyme whereas none was formed by strain 476. The activity of strains 456 and 462 fell between these extremes. Therefore, strain 450 was chosen for further studies.

Effect of growth conditions on extracellular enzyme production. The optimum incubation period for extracellular proteinase production was determined by assaying cell-free growth media after incubation periods of 2, 3, 4, 6, 8, 10, and 12 days. As can be seen in Figure 1, the proteinase concentration increased

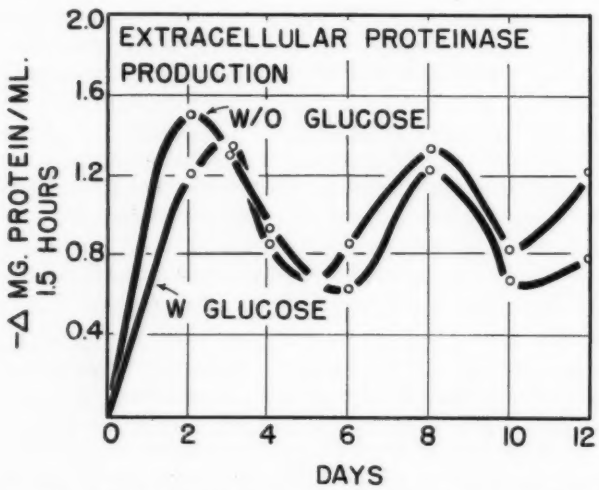

FIG. 1. Extracellular proteinase production. *B. linens* was grown with aeration at 25° C. with and without 0.5% glucose. Assays for proteolytic activity in the cell-free medium were performed at times indicated. Reaction mixtures containing 4.0 ml. of enzyme, 3.0 ml. of 2.5% casein, and 3.0 ml. of borate-phosphate buffer, pH 8.1, were incubated 1½ hours at 38° C.

rapidly, reached a maximum in 2 days, decreased between 2 and 6 days, and reached another maximum in 8 days. The turbidity of the cultures reached a maximum in 3 days and declined gradually thereafter (Figure 2). Since glucose did not influence proteinase production or growth appreciably, the extracellular preparations used in additional studies were made from the medium of 2-day-old cultures grown without glucose.

Proteinase production was greater in a medium originally adjusted to pH 7

than in media set at pH 6 or 8. Although growth was proportional to the amount of aeration, the amount of proteinase formed per milligram of cell nitrogen was independent of the degree of aeration.

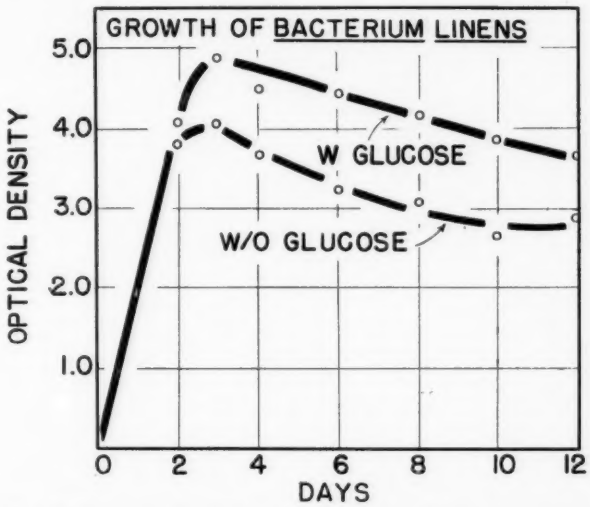

FIG. 2. Growth of *Bacterium linens*, strain 456. The growth conditions were the same as in Figure 1. At the times indicated, the growth in 1.0 ml. of culture, diluted 1:10, was read in an Evelyn colorimeter with a 660 mμ filter. Values expressed as optical density of culture × 10.

Ammonium sulfate fractionation. A concentrated enzyme preparation, free of amino acids and other materials present in the growth medium, was obtained by simple ammonium sulfate fractionation. Preliminary experiments showed that the activity was precipitated in the 52 to 68 and 68 to 100% saturated ammonium sulfate fractions. Almost no activity was found in the lower fractions. Table 1 gives typical results of such a fractionation. Specific activity measurements on the culture medium were not made because of the low concentration of protein. The concentrated preparation, however, had a specific activity in the range of 23.3 to 78.7 mg. of casein disappearing in 1½ hours per milligram of enzyme protein.

End products. The activity of the concentrated preparation was tested by the ninhydrin method, which is capable of detecting the presence of as little as 1.5 γ of α-amino nitrogen. In such assays, an increase in color corresponding to the observed loss in protein was not noted, however. On this basis, the formation of a TCA-soluble polypeptide, without appreciable ninhydrin reaction, was suspected. The TCA-soluble material produced by enzyme action was hydrolyzed with HCl and again subjected to the ninhydrin test. A great increase in color was observed, indicating that polypeptide hydrolysis had occurred. In addition, the TCA filtrate gave a positive biuret reaction which disappeared upon the acid hydrolysis.

TABLE 1

Ammonium sulfate fractionation. Ammonium sulfate (69.7 g./100 ml.) was added to cell-free medium at 4° C. with agitation. The precipitate was dissolved in 1/20 the original volume of distilled water and dialyzed against tap water 4° C. for 20 hours

	Step	Tot. act.[a]	Recov.
		(mg.)	*(%)*
I	Growth medium	1,441	100.0
II	100% Satd. $(NH_4)_2SO_4$[b] (10X conc. undial.)	1,361	94.4
III	II Dial.	871	60.4
IV	III 0-52% Satd. (2X conc. undial.)	0	0
V	IV Dial.	95	6.6
VI	III 52-100% Satd. (2X conc. undial.)	1,061	73.6
VII	VI Dial.	950	65.9

[a] Casein digested in 1.5 hr.
[b] Saturation = 69.7 g./100 ml. of solution.

Quantitative measurements of this proteolytic process may be seen in Table 2. Casein was incubated either with the extracellular enzyme, the cell extract, or both enzyme preparations combined. The extracellular enzyme caused a loss of 3.5 mg. of casein in 6 hours, as measured by the biuret method. In the same time interval, TCA-soluble, biuret-positive material equivalent to 1.99 mg. of casein was formed. The discrepancy between the loss in protein and the increase in TCA-soluble substances may be due to the different absorption maxima of biuret complexes produced by proteins and polypeptides and to a loss of biuret-positive linkages by proteolysis. The ninhydrin values indicate that relatively few amino acids and lower peptides were formed. This was verified by paper chromatography. Upon acid hydrolysis, however, the 0.44 mg. of α-amino nitrogen increased by 1.46 mg.; this value approximated the amount of TCA-soluble biuret material present. The loss in protein and the increase in TCA-soluble nitrogen, as estimated by the absorption at 280 mμ., were essentially in agreement with that measured by the biuret method. In contrast, little proteolytic activity was displayed by the cell-extract enzymes.

When both enzyme preparations together were incubated with casein, the results were approximately the sum of the individual enzyme activities, i.e., 4.00 mg. of casein disappeared and 2.43 mg. of polypeptide (casein equivalent) were formed. The polypeptide was acid-hydrolyzed to non-biuret-positive material. Further evidence for the formation of a polypeptide by the extracellular enzyme was supplied by comparison of paper chromatograms of the unhydrolyzed and hydrolyzed TCA-soluble material. Before hydrolysis, 1 spot of R_f 0.78 was present. After hydrolysis 6 intense spots at R_f 0.20, 0.24, 0.37, 0.60, 0.72, and 0.78 were observed.

TABLE 2

Proteinase activity of enzymes from Bacterium linens. The reaction mixture contained either 1.2 ml. of extracellular enzyme, 4.8 ml. of cell extract, or both enzymes as indicated, and 6.0 ml. of 6.6 per cent casein, 6.0 ml. of borate-phosphate buffer, pH 8.1, and water to 24.0 ml. These were incubated 6 hours at 38° C. and aliquots added to TCA

Enzyme preparations	Δ mg./ml. in 6 hours						
	—TCA—insoluble		+TCA—soluble				
	Casein		Polypeptides		Peptides, amino acids		
			Biuret		Ninhydrin		
	Biuret	280 mμ	Untreated	Acid hyd.	Untreated	Acid hyd.	280 mμ
Extracellular	3.50	2.47	1.99	—	0.44	1.90	1.72
Cell extract	0.80	0.75	0.07	0.07	0.03	0.23	0.44
Extracellular + cell extract	4.00	4.25	2.43	0.09	0.60	2.76	4.41

In view of the high TCA-soluble biuret values and low ninhydrin values after incubation with the combined preparations, it was concluded that the enzymes in the cell extract did not attack the polypeptide material produced by the extracellular enzyme. Whether this was due to a lack of enzyme activity on this polypeptide or due to the low polypeptide concentration was not known. In order to test more directly if the polypeptide could act as a substrate for the cell enzymes, this material was prepared in large quantity, as described under *Methods*. When this preparation was used as a substrate (11.2 mg. per milliliter), the cell extract caused a 16% hydrolysis in 3 hours. Further incubation did not increase this value. In confirmation of the foregoing observations, the extracellular proteinase did not attack this preparation.

Distribution of proteolytic activity. As already shown in Table 2, the extracellular preparation contained a major portion of the proteinases and little activity was observed in the cell extracts. The activity of the enzyme from the growth medium, prepared by ammonium sulfate precipitation, was 50-fold greater than that of the cell extract on the basis of the protein content.

Peptidase activity was determined qualitatively, utilizing paper chromatography to show the amino acids liberated from the peptides by hydrolysis. The extracellular preparation hydrolyzed alanylphenylalanine and leucyltyrosine slowly but did not attack glycyl peptides such as glutathione, glycylglycylglycine, and glycyltyrosine. The cell extracts were somewhat more active on peptides, but the rate of hydrolysis also was of a low order.

Properties of the proteinase. Proteolysis by the cell-free growth medium and the concentrated enzyme continued linearly at a rapid rate for about 4 hours and diminished rapidly, thereafter. In subsequent assays, the incubation period, therefore, was limited to 1½ hours.

Appreciable activity was observed over a pH range of 5.6 to 8.8. When proteolysis was determined by the amount of ninhydrin-positive material released from the TCA filtrate by acid hydrolysis, the optimum pH for proteinase activity was approximately 7.2 to 7.3. A temperature optimum at about 38° C. was

observed. The activity decreased sharply above and below this temperature, but slight proteolysis was evident at 0° C. and at 60° C.

Figure 3 illustrates the effect of increasing the substrate concentration. The rate of proteolysis increased with increasing casein concentration and reached a maximum with approximately 40 mg. of casein per milliliter. In this experiment, the extent of proteolysis was estimated by acid hydrolysis and ninhydrin determinations as above. For rate measurements, a nonlimiting casein concentration was used (60 to 66 mg. per milliliter).

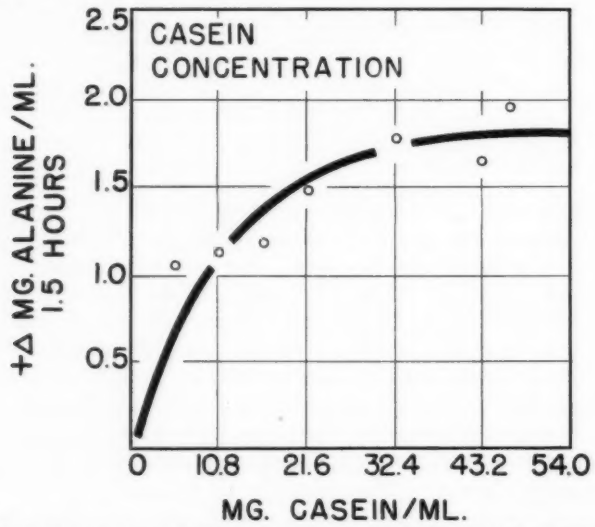

FIG. 3. Effect of casein concentration upon rate of proteolysis. Reaction mixtures containing 0.2 ml. of precipitated enzyme, 6.4% casein as indicated, 1.5 ml. of borate-phosphate buffer, pH 8.1, and water to 6.0 ml. were incubated 1½ hours at 38° C. Results are expressed as alanine (ninhydrin) released by acid-hydrolyzing aliquots of the TCA-soluble material.

The rate of proteolysis as a function of enzyme concentration is shown in Figure 4. The rate rose rapidly at first, but with greater than 0.1 ml. of enzyme (a decrease of 1.3 mg. casein per 1½ hours) the rate increased slowly with the addition of more enzyme. The reason for this is not apparent, since less than 9% of the casein, present in unlimiting concentration, was utilized. The addition of the polypeptide end product (7.4 mg. per milliliter) did not decrease the reaction rate, thus indicating that the decrease in enzyme action was not due to accumulation of this end product.

It has been shown that many proteinases require metallic ions (19, 22) and reducing agents (5, 9, 10) for maximum activity. However, the proteolytic activity of the dialyzed preparation was not affected appreciably by the presence of either 6.6×10^{-4} *M* metallic salts (Fe^{++}, Fe^{+++}, Mn^{++}, Mg^{++}, Mo^{+++}, Ca^{++}, Ni^{++}, Zn^{++}, Cu^{++}, or Co^{++}) or 6.6×10^{-4} *M* reducing agents (ascorbic acid, cysteine, $Na_2S_2O_4$, KCN, $K_4Fe(CN)_6$, $SnCl_2$, or sodium thioglycolate). The lack of ion stimulation

may be due to the fact that sufficient ions were present, contributed either by the tap water used in dialysis or by the casein preparation.

Substrate specificity. To determine the substrate specificity of the extracellular proteinase, the ability to hydrolyze bovine serum albumin, bovine γ-globulins, β-lactoglobulin, bovine seminal proteins, and lactalbumin was tested. In addition to casein (loss of 1.22 mg. per milliliter in 1½ hours), the enzyme attacked only the seminal proteins; however, at a much slower rate (loss of 0.24 mg. per milliliter in 1½ hours). Partial denaturation by gentle heating in warm water increased this value to a loss of 1.12 mg. per milliliter in the same period of time.

Several casein preparations were tested to determine the effect of different methods of preparing casein on the proteinase activity. The casein from raw skimmilk was separated by centrifugation for 2 hours at 40,000 × gravity and resuspended in buffer with a small amount of *N* NaOH. This preparation was relatively undenatured compared to casein prepared commercially by acid precipitation and heat drying. As can be seen in Figure 5, the action on crude

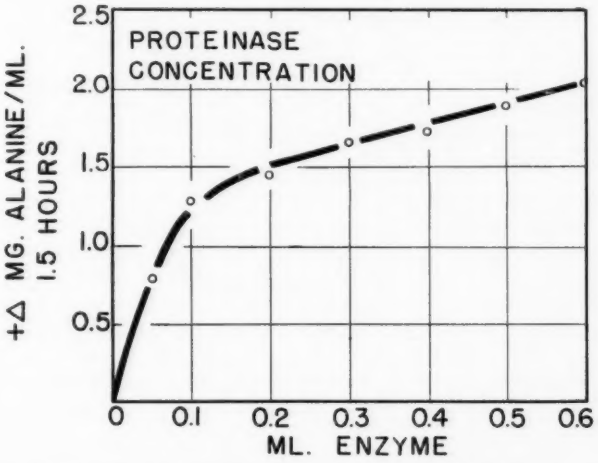

FIG. 4. Effect of the proteinase concentration upon the rate of proteolysis. The conditions were the same as in Figure 2, except that graded amounts of enzyme added and 1.5 ml. of 6.4% casein were used.

centrifuged casein was considerably greater than that on the GBI casein. The results may be due to the comparatively undenatured state of the casein from skimmilk or possibly to the presence of unknown activators in such a preparation.

Inasmuch as casein is not homogenous, but is composed of α, β, and γ fractions (11), the ability to hydrolyze individual fractions was tested. As shown in Figure 5, both the α and β fractions were hydrolyzed. The rate of α-casein hydrolysis was intermediary between that of centrifuged casein and the GBI casein. The initial rate of protein disappearance with β-casein almost paralleled

that of the α preparation; however, proteolysis ceased at approximately 2½ to 3 hours, whereas the hydrolysis of the α fraction continued at a slow rate. Also, as hydrolysis of β-casein proceeded, the turbidity of the reaction mixture increased until the solution was opaque (4 hours). The reaction causing the turbidity increased at a rapid rate beyond 6 hours, even though the hydrolysis, as measured by protein disappearance, had stopped at 3 hours. The development of turbidity was not inhibited by pretreating the β-casein with oxalate for 20

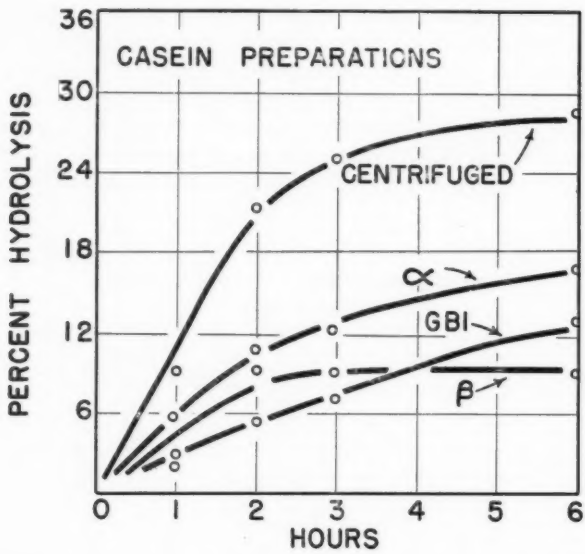

FIG. 5. Proteinase action on casein preparations. Reaction mixtures containing 0.2 ml. of enzyme, 1.5 ml. of 6.5% casein (GBI), or 3.0 ml. of either 2.0% casein (centrifuged), 3.0% α-casein, or 3.0% β-casein, 1.5 ml. of borate-phosphate buffer, pH 8.1, and water to 6.0 ml. were incubated at 38° C. and aliquots removed at times indicated for protein determinations.

hours or by adding oxalate to the reaction mixture. Inorganic phosphate or arsenate was necessary for production of the turbidity, but not for proteolysis. β-casein incubated without enzyme or with heat-inactivated enzyme for 15 hours at 38° C. showed no turbidity. None of the other casein preparations or proteins tested displayed this characteristic.

DISCUSSION

The proteinase production which occurred before 2 days and between 6 and 8 days seems not to be the result of cell autolysis. The activity appearing in the first 2 days was produced while the culture was in the logarithmic growth phase, during which little autolysis occurs, and the enzyme present in 8 days was produced during the stationary phase in which there is no corresponding autolysis

(Figures 1 and 2). The proteinase present after 2 days of growth is typical of bacterial proteinases (21, 22) but may be different from that produced between 6 and 8 days. The latter enzyme was not studied, however. Thomasow (20) observed a similar fluctuation in the enzyme production with maxima at 2 and 21 days in cultures grown without aeration, but changes in cell numbers were not measured.

The decreased rate of hydrolysis, after 6 to 10% degradation of casein has occurred (Figure 2), is not due to inhibition by the end products (4) or to a limiting substrate concentration in the usual sense. Simple enzyme inactivation also seems unlikely, since only slight additional hydrolysis occurs over a 6-fold increase in enzyme concentration (Figure 3). There are, however, at least two possible explanations for the inability to effect further hydrolysis.

It is possible that all of the substrate was attacked, in that 5 to 10% of the biuret-positive linkages was hydrolyzed from all of the casein molecules, forming TCA-soluble polypeptides and leaving an unaltered TCA-soluble substance which is no longer subject to proteinase attack. This would be compatible with terminal group specificity displayed by certain proteinases (18) and also consistent with the resistant "core" hypothesis mentioned by Balls and Jansen in a review on proteolytic enzymes (2).

The action of the extracellular proteinase on β-casein may offer some explanation for the abrupt decrease in the rate of hydrolysis. The results of such action suggest that there may be two phases of the proteolytic action by the extracellular preparation and that the action of the first (casein disappearance) is halted by the increasing effect of the second (precipitation of β-casein). It is possible that unfolding and rupture of peptide chains of the casein molecule during proteolysis would render it resistant to further proteolytic attack. While β-casein was the only protein tested which displayed a visual alteration of the molecule by the extracellular enzyme, a similar attack without an increase in turbidity may occur with α- and γ-casein.

CONCLUSION

An extracellular proteinase (sp. act. 23.3 to 78.7 mg. of casein hydrolyzed in 1½ hours per milligram of enzyme protein) has been recovered from the medium after 2 days' growth of *Bacterium linens*, 450, by ammonium sulfate precipitation (52 to 100% saturation). Of the proteins tested, only casein and a bovine seminal protein preparation were attacked. The product of proteinase action is a polypeptide. No polypeptidase and little peptidase activity was found in the proteinase preparation. With β-casein as the substrate, a proteolytic action superficially similar to that of rennin was observed.

REFERENCES

(1) Albert, J. O., Long, H. F., and Hammer, B. W. Classification of the Organisms Important to Dairy Products. IV. *Bacterium linens*. Iowa Agr. Expt. Sta. Research Bull. 328. 1944.

(2) Balls, A. K., and Jansen, E. F. Proteolytic Enzymes. Ann. Rev. Biochem., 21: 1-28. 1952.

(3) CONSDEN, R., GORDON, A. H., AND MARTIN, A. J. P. Qualitative Analysis of Proteins: A Partition Chromatographic Method Using Paper. Biochem. J., **38**: 224-232. 1944.

(4) FRASER, D., AND POWELL, R. E. The Kinetics of Trypsin Digestion. J. Biol. Chem., **187**: 803-820. 1950.

(5) VAN HEYNINGEN, W. E. The Proteinases of *Clostridium histolyticum*. Biochem. J., **34**: 1540-1545. 1940.

(6) IYA, K. K., AND FRAZER, W. C. The Yeast in Surface Smear of Brick Cheese. J. Dairy Sci., **32**: 475-476. 1949.

(7) JOHNSON, B. C., AND SWANSON, A. M. Milk Serum Proteins. I. A Quantitative Biuret Test for Milk Serum Proteins. J. Dairy Sci., **35**: 823-828. 1952.

(8) KELLY, C. D., AND MARQUARDT, J. C. The Influence of Hydrogen Ion Concentration and Salt on the Surface Flora of Limburger Cheese. J. Dairy Sci., **22**: 309-320. 1938.

(9) MASCHMANN, E. Zur Kenntnis der Proteasen des *B. sporogenes*. Über eine neue Proteinase und über aktivierbare Peptidasen. Biochem. Z., **300**: 89-112. 1939.

(10) MASCHMANN, E. Über Bakterienproteasen. XVI. Zur Kenntnis der Aminopolypeptidasen anaerober Bakterien. Biochem. Z., **307**: 1-34. 1940.

(11) MELLANDER, O. Elektrophoretische Untersuchung von Casein. Biochem. Z., **300**: 240-245. 1939.

(12) MOORE, S., AND STEIN, W. H. Photometric Ninhydrin Method for Use in Chromatography of Amino Acids. J. Biol. Chem., **176**: 367-388. 1948.

(13) PURKO, M., NELSON, W. O., AND WOOD, W. A. The Associative Action Between Certain Yeasts and *Bacterium linens*. J. Dairy Sci., **34**: 699-705. 1951.

(14) ROBINSON, H. W., AND HOGDEN, G. G. The Biuret Reaction in the Determination of Serum Proteins. I. A Study of the Conditions Necessary for the Production of a Stable Color which Bears a Quantitative Relationship to the Protein Concentration. J. Biol. Chem., **135**: 707-725. 1940.

(15) SHULL, G. M., HUTCHINGS, B. L., AND PETERSON, W. H. A Microbiological Assay for Biotin. J. Biol. Chem., **142**: 913-920. 1942.

(16) SNOKE, J. E., AND NEURATH, H. The Proteolytic Activity of Striated Rabbit Muscle. J. Biol. Chem., **187**: 127-135. 1950.

(17) STEINFATT, F. Über *Bacterium linens* und Seine Beziehungen zu einigen seiner Begleitorganismen in der Käserotschmiere beim Eieweisabbau in Milch. Milchwirtsch. Forsch., **9**: 1-50. 1930.

(18) SUMNER, J. B., AND MYRBÄCK, K. *The Enzymes*. Vol. 1, part 2. Academic Press, Inc., New York. 1951.

(19) STOCKTON, J. R., AND WYSS, O. Proteinase Production by *Bacillus subtilis*. J. Bacteriol., **52**: 227-228. 1946.

(20) THOMASOW, J. Über Enzyme des *Bakterium linens*. Kieler Milchwirt. Forschberichte., **2** (1): 35-62. 1950.

(21) VIRTANEN, A. J., AND TARNANEN, J. Die Sekretion und Thermostabilität der Bakterienprotiasen. Z. physiol. chem., **204**: 247-258. 1932.

(22) WEIL, L., AND KOCHOLATY, W. Studies on the Proteinase of *Clostridium histolyticum*. Biochem. J., **31**: 1255-1267. 1937.

(23) WOOD, A. J., AND GUNSALUS, I. C. The Production of Active Resting Cells of Streptococci. J. Bacteriol., **44**: 333-341. 1942.

DISTILLERS FEEDS AS PROTEIN SUPPLEMENTS IN CONCENTRATE MIXTURES FOR MILKING COWS

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The results of experiments comparing corn distillers dried grains with solubles, corn distillers dried solubles, rye distillers dried grains with solubles, and a mixture of corn gluten feed and solvent extracted soybean oil meal as the principal protein supplement to barley and oats for milking cows have recently been published by Loosli *et al.* (3). From these studies the authors suggested that the corn gluten feed-soybean oil meal mixture was not as efficient for milk production as was the corn distillers dried grains with solubles. The corn distillers dried solubles and rye distillers dried grains with solubles were essentially equal to the corn gluten feed-soybean oil meal combination. Since the corn distillers dried grains with solubles were somewhat, though not significantly, better than corn distillers dried solubles, it seemed important to expand the study and compare directly corn distillers dried grains with these other distillers feeds.

EXPERIMENTAL

The three concentrate mixtures, using distillers feeds as the principal protein supplement to barley and oats, are shown in Table 1. They were adjusted

TABLE 1
Composition of the concentrate mixtures

Ingredients	Mixture No. I	II	III
	(lb.)	*(lb.)*	*(lb.)*
Barley	283	283	283
Oats	252	252	252
Molasses	50	50	50
Corn distillers dried grains with solubles [b]	400	.....	.....
Corn distillers dried grains [b]	.....	400	.....
Corn distillers dried solubles [b]	.....	.....	400
Dicalcium phosphate	10	10	10
Salt	5	5	5
Total [a]	1000	1000	1000

[a] Plus 1 g. of cobalt sulfate per mixture.
[b] From a mash bill of 91% corn and 9% barley malt.

to contain approximately 18% of total protein. In addition to the concentrate mixtures, the cows were fed well-eared corn silage and either a U. S. No. 2 timothy heavy grass mixed hay or a U. S. No. 2 mixed hay containing a small proportion of birdsfoot trefoil. The timothy hay was fed to all cows during the first third of the experiment, but, because of the limited supply, the cows

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were shifted to the mixed hay containing some legumes during the latter two-thirds of the study. The chemical analyses of the various feeds used in the experiment are shown in Table 2.

TABLE 2
Chemical analyses of the feeds

Material	Moisture	Protein	Ether extract	Fiber	N.F.E.	Ash
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Corn distillers dried grains with solubles	5.3	25.9	13.7[c]	11.9	39.8	3.4
Corn distillers dried grains	6.2	29.5	15.1[c]	15.1	32.4	1.7
Corn distillers dried solubles	8.8	27.6	13.0[c]	6.1	36.4	8.1
Concentrate mixtures containing:						
Corn distillers dried grains with solubles	10.2	18.1	6.4	7.7	53.0	4.6
Corn distillers dried grains	10.2	17.7	6.4	7.7	53.2	4.8
Corn distillers dried solubles	9.2	18.1	6.4	5.4	53.4	7.5
Corn silage, well-eared	77.2	3.6	1.1	9.6	6.6	1.9
Timothy hay[a]	8.3	6.2	1.5	35.4	43.7	4.9
Mixed hay[b]	8.0	8.4	1.8	36.5	40.3	5.0

[a] U. S. No. 2 timothy heavy grass mixed hay fed during first third of the study.
[b] U. S. No. 2 mixed hay containing 60-80% timothy, 15% birdsfoot trefoil and the remainder mixed grasses and foreign material fed during last two-thirds of the study.
[c] Analyzed without previous water extraction. Later samples from the same mashes contained 11.6% of ether extract for dried grains, 12.4% for dried grains with solubles and 6.7% for dried solubles following water extraction.

Twelve purebred Holstein cows which had recently passed their maximum milk production but had not been rebred were selected for the study. They were fed the timothy hay and corn silage plus a commercial 18% total protein concentrate mixture during a preliminary period of at least 4 weeks to observe levels of production and to adjust feed intakes. The cows were then divided into four replicates on the basis of production and assigned at random to a 3 × 3 Latin square design. At the beginning of the preliminary period, hay and corn silage were fed at the approximate rates of 1 and 3 lb. per 100 lb. of body weight, respectively. During the experimental period the amount of silage offered was kept constant and with one or two exceptions was completely consumed. Sufficient hay was fed so that the cows left 1 to 3 lb. of hay per day. At the start of the experiment, the concentrate mixture was fed at the rate of 1 lb. to each 3.5 lb. of fat-corrected milk. The rate of grain feeding was reduced each week in accordance with the average decrease in production of all cows as described by Lucas (4). The experimental periods were 6 weeks in duration. The first week was considered a transition period, and milk production and feed intake data were accumulated during the last 5 weeks of each experimental period.

RESULTS AND DISCUSSION

The average daily production of milk, fat, and fat-corrected milk, and the average fat percentages are summarized in Table 3. An analysis of variance of the individual data showed that the differences in fat percentage on the

TABLE 3
The average daily milk and fat production

Mixture containing	Milk	Fat	Fat	FCM[a]
	(lb.)	*(%)*	*(lb.)*	*(lb.)*
Corn distillers dried grains with solubles	32.0	3.58	1.15	30.0
Corn distillers dried grains	33.2	3.65	1.21	31.4
Corn distillers dried solubles	32.8	3.45	1.13	30.1

[a] Fat-corrected milk (Gaines, 1).

three different rations approached significance at the 5% level (odds = 18:1). This suggests that in this experiment the milk tested slightly higher when the cows were fed corn distillers dried grains than when they were fed the concentrate mixtures containing corn distillers dried solubles. The differences in pounds of milk, or in pounds of fat-corrected milk were not statistically significant, although there was a slight actual advantage for the periods when corn distillers dried grains were fed.

The average feed intakes, the body weight of the cows, and the average daily gain in weight are summarized in Table 4. Except for a small difference

TABLE 4
The average daily feed intakes and changes in body weight

Mixture containing	Concentrate	Silage	Hay	Body weight	Weight gain
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
Corn distillers dried grains with solubles	13.7	39	5.2	1178	0.4
Corn distillers dried grains	13.7	39	6.8	1174	0.5
Corn distillers dried solubles	13.7	39	7.2	1178	0.4

in the amounts of hay consumed, the feed intakes and the gain in weight of the cows were very nearly the same on the different experimental feeds.

In studies reported previously (Loosli *et al.*, 3), there was no difference in the amount of concentrates or silage required, but slightly less hay was used to produce each 100 lb. of fat-corrected milk with the corn distillers dried grains with solubles than with the corn distillers dried solubles. In the current study it is seen (Table 5) that slightly less concentrate mixture, silage, and hay were required to produce 100 lb. of fat-corrected milk when corn distillers dried

TABLE 5
Data obtained from lactating dairy cows consuming three distillers feeds

Mixture containing	Av. daily FCM[a]	Av. fat percentage	Feed per 100 lb. FCM[a]			Gain in weight
			Concentrate	Silage	Hay	
	(lb.)	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
Corn distillers dried grains with solubles	32.0	3.58	45.7	129	17.3	1.5
Corn distillers dried solubles	32.8	3.45	45.5	128	23.9	1.3
Corn distillers dried grains	33.2	3.65	43.6	124	21.7	1.7

[a] Fat-corrected milk (Gaines, 1).

grains furnished the protein in the concentrate mixture than when corn distillers dried solubles were used. These differences are extremely small but probably can be accepted as reliable evidence that corn distillers dried solubles are not more valuable than corn distillers dried grains for dairy cows. It would appear, therefore, that the high B vitamin content of the solubles, although important in nonruminant feeding, has no unusual value for milking cows fed liberal amounts of hay and corn silage.

The percentage of ether extract material in distillers feeds (Table 2) deserves further comment. It has been shown by Gustoff and Rasmussen (2) that certain samples of distillers dried solubles require water extraction before accurate ether extract determinations can be made. This treatment was not necessary with distillers dried grains. The data in Table 2 were obtained using conventional techniques without previous water extraction. The values for the feeds containing the distillers dried solubles, therefore, are probably erroneously high. The reason for the high concentration of ether extract in the distillers dried grains is not readily apparent since similar analyses of other samples of a similar product generally have not yielded results of this magnitude. If this lot of distillers dried grains actually contained 15.1% true fat, it may have been important in this study (Maynard *et al.*, 5). Unfortunately, it was not possible to check the analyses because the samples had been completely used.

These data confirm previous studies as to the value of corn distillery by-products when used to make up as much as 40% of the concentrate mixture for milking cows.

SUMMARY

An experiment comparing corn distillers dried grains, corn distillers dried grains with solubles, and corn distillers dried solubles was conducted with lactating dairy cows.

A slight though not statistical advantage was observed for corn distillers dried grains over both corn distillers dried solubles and corn distillers dried grains with solubles for the efficiency of production as well as the total production of fat-corrected milk. Corn distillers dried grains increased the fat percentage of the milk slightly. The data confirm previous studies as to the usefulness of corn distillery by-products when used to make up as much as 40% of the concentrate mixture. It appears that the corn distillers dried solubles, although extremely useful in nonruminant feeding, do not have a unique value for lactating dairy cows fed liberal amounts of corn silage and hay.

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REFERENCES

(1) GAINES, W. L. The Energy Basis of Measuring Milk Yield in Dairy Cows. Ill. Agr. Expt. Sta. Bull. 308. 1928.

(2) GUSTOFF, F., AND RASMUSSEN, R. A. Referee Methods for Determination of Moisture and Fat on Distillers Feeds. Proc. Eighth Distillers Feed Conference, p. 14. 1953.

(3) LOOSLI, J. K., TURK, K. L., AND MORRISON, F. B. The Value of Distillers Feeds for Milk Production. J. Dairy Sci., **35**: 868-873. 1952.

(4) LUCAS, H. L. A Method of Equalized Feeding for Studies with Dairy Cows. J. Dairy Sci., **26**: 1011. 1943.

(5) MAYNARD, L. A., MCCAY, C. M., WILLIAMS, H. H., AND MADSEN, L. L. II. Further Studies of the Influence of Different Levels of Fat Intake upon Milk Secretion. Cornell Agr. Expt. Sta. Bull. 593. 1934.

RELATIVE VALUE FOR MILK PRODUCTION OF FIELD-CURED AND FIELD-BALED, ARTIFICIALLY DRIED-CHOPPED, ARTIFICIALLY DRIED-GROUND AND ARTIFICIALLY DRIED-PELLETED ALFALFA WHEN FED AS THE SOLE SOURCE OF ROUGHAGE TO DAIRY CATTLE [1]

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Artificial dehydration and pelleting apparently provide a method whereby the quality and nutritive value of green alfalfa may be preserved and shipping costs decreased. Relatively high levels of nutrient preservation in curing hays by artificial drying have been demonstrated by numerous workers (12, 15, 16, 17, 23, 24, 25). Further, it has been shown that artificial drying apparently has no deleterious effect upon the availability of the hay nutrients (8). Grinding has, in some instances, improved the digestibility of hay. Although the digestibility of protein, fat, ash, and nitrogen-free extract may be increased somewhat by grinding, that of crude fiber is usually lowered (1, 2, 15). It has been shown (1) that the lower digestibility of crude fiber in ground hay is associated with the dry matter content of the rumen ingesta, rather than the rate of passage of the hay through the reticulo-rumen. When dehydrated chopped, finely-ground, and pelleted alfalfa hays were fed at a 30% grain replacement level (3), the cows produced significantly greater amounts of fat-corrected milk (FCM) on the pelleted alfalfa than on finely-ground alfalfa.

In view of the apparent benefits derived from artificially drying and pelleting, this study was conducted to determine the relative value for milk production of alfalfa hay in field-cured and field-baled, artificially dried-chopped, artificially dried-ground, and artificially dried-pelleted forms when fed as the sole source of roughage.

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EXPERIMENT I

Animals. During the period January-August 1950, eighteen cows from the University of Connecticut dairy herd were used in the experiment. They were divided into six groups of three cows each, four of Guernseys and two of Holsteins. The animals in each group were balanced as nearly as possible with respect to age, stage of lactation, weight, and milk production.

Feeds. The three forms of alfalfa were harvested from a field estimated to be in one-quarter bloom when cut in Dawson County, Nebraska, in the Platte River Valley. The alfalfa for artificial drying and processing into meal and pellets was cut with a field harvester and immediately hauled to dehydrators. The alfalfa for field-curing and baling was cut the same day and was cured and baled during a subsequent 5-day period. Some of the baled hay was rained on during the curing process. The pellets were cylindrical in shape, 1.75 cm. in diameter and on the average 2.2 $\pm$ 0.6 cm. in length.

It was noted that the artificially dried-ground alfalfa was exceedingly dusty and apparently lacking in palatability. In preliminary trials, it was observed that the ground alfalfa was not being consumed at as high a level as the baled and pelleted hays. Therefore molasses was added at the rate of 10% by weight to the ground form to decrease the excessive dustiness and increase palatability to a point where total intake would be more nearly comparable to that of the baled and pelleted alfalfa.

The grain mixture fed contained per ton 900 lb. of crimped oats, 700 lb. hominy feed, 220 lb. standard wheat bran, 100 lb. linseed oil meal, 40 lb. salt, and 40 lb. steamed bone meal. The average chemical composition of feeds as fed is given in Table 1.

TABLE 1

The average chemical composition of feeds as fed

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	N.F.E.	Ash
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Field-cured and field-baled alfalfa	89.3	16.9	1.3	28.9	33.0	9.3
Artificially dried-ground alfalfa	90.1	15.7	2.0	22.8	40.3	9.4
Artificially dried-pelleted alfalfa	90.0	17.5	2.8	24.3	36.0	9.5
Grain ration	92.2	12.9	4.3	7.8	60.4	7.9

Procedure. Each form of alfalfa was fed for a 40-day period, using a double change-over design (4). The data collected during the initial 10 days of each period were not utilized in calculating results.

The hays were fed ad lib at a rate sufficient to provide a 5 to 10% weigh-back each day. One lb. of grain was fed for each 4 lb. FCM produced. Grain levels were recalculated at the end of each 10-day period, using the previous 30 days'

production data as a basis for calculation. All feeds fed were weighed individually for every cow, as were weigh-backs at each feeding. Composite samples of the roughage fed, as well as individual cow refusals, were taken for each 30-day experimental period. A composite sample of the grain was obtained by sampling every fifth bag during mixing. Chemical analyses [4] were determined on all feed samples throughout the trials. Feed consumption in terms of total digestible nutrients was calculated by applying digestion coefficients listed in Morrison's tables (13) to the chemical analyses of feeds fed and refused.

The milk from each cow was weighed at each milking and a 2-day composite milk sample was taken for each cow the 5th and 6th days of each 10-day period. All cows were weighed for three consecutive days at the beginning and end of each 30-day experimental period. In analyzing for variance, the procedure followed was that proposed by Cochran *et al.* (4).

RESULTS AND DISCUSSION

Feed consumption. The total dry matter and digestible nutrient intake from roughage and grain combined (Table 2) was essentially the same for each form of alfalfa. Preference by individual cows for a particular form of alfalfa resulted

TABLE 2
Feed consumption as affected by form of alfalfa fed

Criteria	Form of alfalfa		
	Baled	Ground	Pelleted
	(lb.)	*(lb.)*	*(lb.)*
Actual:			
Roughage	14,772	15,354	15,283
Grain	5,710	5,494	5,340
Total	20,482	20,848	20,623
Dry Matter:			
Roughage	13,111	13,841	13,719
Grain	5,226	5,065	4,856
Total	18,377	18,906	18,575
Digestible Nutrients:			
Roughage	7,269	7,860	7,741
Grain	4,049	3,897	3,786
Total	11,318	11,757	11,527

in different, though not significant, levels of roughage intake. These differences, however, were compensated for to a considerable degree by varying amounts of grain consumption which were significantly higher (TDN, $P < 0.05$; DM, $P < 0.01$) when the cows were fed field-baled alfalfa.

A tendency toward an inverse relationship between roughage and grain consumption was evident (Table 2). Total grain refusals for all cows amounted

[4] Samples for chemical analysis were sent to the Connecticut Agricultural Experiment Station at New Haven and analyzed for percentage of moisture, ash, crude protein, crude fiber, nitrogen-free extract, and ether extract.

to 26.1, 146.0, and 295.0 lb., respectively, for baled, ground, and pelleted hay. The higher grain refusals could have been due to larger intakes of ground and pelleted hays and/or to a similarity in the concentrated physical state of grain and those forms of hay and a consequent diminished appetite for grain.

Milk production. Data regarding milk yields, presented in Table 3, showed actual milk production to be lower ($P < 0.01$) when the cows were fed field-baled alfalfa. Since these lower yields, however, were significantly higher ($P <$ 0.01) in fat content, there was no appreciable difference in ration effects upon FCM. These results only partially confirm those of Powell (18), who reported lower fat content of the milk without any effect on quantity from feeding both the roughage and grain in the compressed state.

TABLE 3
Total milk and fat yields as affected by form of alfalfa fed

Criteria	Form of alfalfa		
	Baled	Ground	Pelleted
Actual milk *(lb.)*	19,433	20,884	21,703
Fat *(lb.)*	792	800	797
Fat *(%)*	4.07	3.83	3.67
FCM *(lb.)*	19,651	20,346	20,641

Average daily milk production in pounds and fat percentage were: for Period I, 43.70, 4.04; for Period II, 38.00, 4.02; and for Period III, 33.20, 4.00. The drop in average daily milk yield for the 18 animals followed the expected trend (4, 5, 7, 19). Fat percentage remained fairly constant within a 0.04% range throughout the three periods. This would normally be expected, since no significant upward trend in fat percentage was observed prior to the 6th or 7th month of lactation (4). The average stage of lactation for all cows at the time of going off experiment was 177 days, or 5.7 months.

Feed sample analyses. Chemical analyses of the hays fed and refused (Table 4) indicated considerable animal sorting of the field-baled alfalfa, for that refused was appreciably lower ($P < 0.01$) in crude protein, ether extract, nitrogen-free extract, and ash content. Conversely, the crude fiber content was higher ($P < 0.01$). Similar data for the ground and pelleted forms showed no significant differences between the roughage fed and that refused. Investigation of individual cow differences (Table 5) indicated that for the baled form these

TABLE 4
Average chemical analyses of feeds fed and refused

Form of alfalfa	Dry matter		Crude protein		Ether extract		Crude fiber		N.F.E.		Ash	
	Fed	Ref.	Fed	Ref.	Fed	Ref.	Fed	Ref.	Fed	Ref.	Fed	Ref.
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Baled	89.3	89.4	16.9	13.2	1.3	0.9	28.9	35.9	33.0	31.2	9.3	8.2
Ground	90.1	89.0	15.7	16.2	2.0	1.9	22.8	22.0	40.3	39.6	9.4	9.4
Pelleted	90.0	89.5	17.5	17.8	2.8	2.8	24.3	24.3	36.0	35.1	9.5	9.5

TABLE 5
Statistical summary of the effect of the form of alfalfa on individual cow differences between that fed and refused

Chemical Constituent	Form of alfalfa		
	Baled	Ground	Pelleted
Crude protein	NS[a]	NS	NS
Ether extract	$P < 0.05$	NS	NS
Crude fiber	NS	NS	NS
N.F.E.	$P < 0.01$	$P < 0.01$	$P < 0.01$
Ash	$P < 0.01$	NS	NS

[a] Nonsignificant

differences were of considerable importance and for the ground and pelleted forms of lesser importance.

Live weight. Live weight averages for the eighteen cows while on each form of alfalfa were for the field-baled 1,116 lb., for the ground, 1,119 lb., and for the pelleted, 1,115 lb. The average live weight of all cows during Period I was 1,115 lb., during Period II, 1,116 lb., and 1,117 lb. in Period III. The fact that live weight was not significantly affected by rations or periods is apparent from the above data.

EXPERIMENT II

In Experiment I, a comparison of the relative value of field-cured and field-baled, artificially dried-ground and artificially dried-pelleted alfalfa hays showed no statistical differences for FCM. Since the field-cured and field-baled hay was rained on during the curing process, the artificially dried-ground hay was so unpalatable that molasses had to be added and the amounts of grain refused were different between ration groupings, a second trial was conducted in an attempt to rectify these deficiencies.

EXPERIMENTAL

Animals. Eighteen Guernsey cows from the University of Connecticut dairy herd during the period January-August, 1951, were used in the experiment. They were divided into six groups of three cows each as described under Experiment I.

Feeds. The three alfalfa hays, considered to be in early bloom when harvested, were grown on similar plots and processed in a manner similar to that previously described under Experiment I. However, the field-cured and field-baled hay was not rained on and required only 3 days from cutting to storage instead of 5 days. The pellets were cylindrical, 1.7 cm. in diameter and 0.9 cm. $\pm$ 0.2 cm. in length.

The grain mixture fed contained per ton 840 lb. of crimped oats, 660 lb. hominy feed, 160 lb. standard wheat bran, 60 lb. linseed oil meal, 200 lb. molasses, 40 lb. salt, and 40 lb. steamed bone meal. The chemical composition of the feeds is given in Table 6.[5]

[5] Loc. cit.

TABLE 6

The average chemical composition of feeds as fed

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	N.F.E.	Ash
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Field-cured and field-baled alfalfa	90.5	17.4	1.7	28.1	34.0	9.4
Artificially dried-chopped alfalfa	91.7	18.0	2.2	25.6	36.2	9.7
Artificially dried-pelleted alfalfa	91.3	18.8	3.4	23.6	34.8	10.9
Grain ration	91.5	11.6	4.4	6.0	62.3	7.3

TABLE 7

Feed consumption as affected by form of alfalfa fed

Criteria	Form of alfalfa		
	Baled	Chopped	Pelleted
	(lb.)	*(lb.)*	*(lb.)*
Actual:			
Roughage	16,359	14,959	10,848
Grain	4,458	4,624	4,562
Total	20,817	19,583	15,410
Dry Matter:			
Roughage	14,755	13,730	9,956
Grain	4,113	4,268	4,207
Total	18,868	17,998	14,163
Digestible Nutrients:			
Roughage	8,167	7,663	5,487
Grain	3,412	3,541	3,468
Total	11,579	11,204	8,954

Procedure. The design and all procedures were identical to Experiment I with the exception of milk sampling. A composite sample was obtained from each cow for each 10-day period by removing an aliquot portion from each milking in an attempt to obtain maximum accuracy in measuring fat production.

RESULTS AND DISCUSSION

Feed consumption. As shown in Table 7, the field-baled and dehydrated-chopped alfalfa hays were consumed at higher levels (DM, $P < 0.01$; TDN, $P < 0.01$) than was the pelleted form.

These data differed from those of Experiment I, which showed little difference in total dry matter and digestible nutrient intake. The low consumption of pellets became more apparent when the feed intake was calculated on the basis of daily consumption per 1,000 lb. of live weight. Consumption was 20.9 lb., in contrast to 27.4 for the chopped and 30.3 for the baled forms, respectively.

Rejection of the pellets was very pronounced in two cases, resulting in such low roughage consumption that the animals showed severe anorexia. Both ani-

mals developed rectal temperatures in excess of 106° F. Faced with possible death, the animals were switched to baled alfalfa in the middle of the 30-day period. The response in increased roughage intake was immediate. Missing values for these two periods were calculated according to standard methods (21).

A partial explanation for the low pelleted-hay intake in the second trial would appear to be the degree of pellet firmness. These differences observed in pellets used in the two trials were confirmed by crushing tests. The average crushing point of 468 lb. per square inch for the pellets fed in the second experiment was significantly higher ($P < 0.02$) than the average crushing point of 205 lb. per square inch for the pellets fed in the first trial.[6]

It was further noted that the particle size of the second trial pellets was smaller. In an attempt to substantiate the latter observation, a 200-g. sample of each type of pellet was placed in a large evaporating dish and covered with water. After the pellets were thoroughly saturated and had completely crumbled, they were placed in a drying oven held at 40-60° C. After evaporation to dryness with heat, they were air-dried for a 24-hour period and then weighed. They then were passed through a series of U. S. standard sieves. The amount remaining in each sieve was weighed to determine the percentage retained. The percentages retained by the various sieves of the pellets used in Experiment I and those in Experiment II, were, respectively, No. 16, 6.7 and 0; No. 20, 17.0 and 0; No. 30, 18.7 and 5.7; No. 40, 16.9 and 12.1, and No. 50, 14.6 and 16.3, with 26.8 and 65.9 passing through No. 50. Apparently the alfalfa in the second experiment was ground more finely and was therefore compressed into a harder pellet. It was believed that the hardness of the pellets was the reason for the lower consumption of the pelleted alfalfa.

Milk production. With the exception of that relating to per cent fat, data relative to production (Table 8) differed from those obtained in Experiment I. Ration differences regarding actual milk production were not significant. However, fat and FCM were greater ($P < 0.01$) when the cows were fed the field-baled and dehydrated chopped alfalfa. In agreement with Powell (18), the feeding of pelleted hay resulted in a significantly lower ($P < 0.01$) percentage of fat in the milk and was similar to that for the first experiment. A plausible explanation for the reduced fat yield, other than a change in the physical state

TABLE 8

Total milk and fat yield as affected by form of alfalfa fed

Criteria	Form of alfalfa		
	Baled	Chopped	Pelleted
Actual milk *(lb.)*	16,328	16,866	16,144
Fat *(lb.)*	693	710	610
Fat *(%)*	4.24	4.21	3.78
FCM *(lb.)*	16,925	17,393	15,702

[6] The crushing tests were performed on a Baldwin Southwork Tensil Testing Machine by courtesy of the U. S. Bureau of Mines, College Park, Md.

of the roughage fed, has been presented by Tyznik and Allen (22). These workers demonstrated a change in the ratio of fatty acid production in the rumen. This change was attributed to a low roughage intake and was accompanied by a depression in fat content of the milk. It was interesting to note that in Experiment I where roughage intake was similar for the three forms of alfalfa fed, fat yields were not significantly different. In the second trial, however, the pelleted hay was consumed at a lower ($P < 0.01$) level than either the field-baled or dehydrated-chopped alfalfa, and fat production was likewise lower ($P < 0.01$).

Milk production and fat percentage for Period I were 35.0 and 4.14; for Period II, 30.3 and 4.11; and for Period III, 26.0 and 4.14. These trends in milk production and fat percentage showed that the drop in average daily milk yield for the 18 animals followed the expected trend (1), while fat percentage remained practically constant, with a 0.03% range throughout the three periods.

Feed sample analyses. Marked animal sorting of the field-baled and dehydrated-chopped alfalfa was observed (Table 9). The crude protein, ether extract, and ash content of the baled hay refused were considerably lower ($P < 0.01$)

TABLE 9
Average chemical analyses of feeds fed and refused

Form of alfalfa	Dry matter		Crude protein		Ether extract		Crude fiber		N.F.E.		Ash	
	Fed	Ref.	Fed	Ref.	Fed	Ref.	Fed	Ref.	Fed	Ref.	Fed	Ref.
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Baled	90.5	90.5	17.4	14.7	1.7	1.3	28.1	32.8	34.0	32.8	9.4	8.9
Chopped	91.7	91.2	18.0	21.7	2.2	2.8	25.6	20.5	36.2	35.1	9.7	11.1
Pelleted	91.3	90.3	18.8	18.7	3.4	3.3	23.6	25.5	34.8	34.4	10.9	10.5

and the crude protein, ether extract, and ash in the chopped alfalfa weigh-backs were higher ($P < 0.01$) than in the roughage as fed. Conversely, the crude fiber was higher ($P < 0.01$) in the field-baled refusals and lower ($P < 0.01$) in the chopped hay refused. Only the baled hay refusals showed significance ($P < 0.05$) with respect to nitrogen-free extract. Similar data for the dehydrated pelleted hay showed no significant difference between the roughage as fed and that refused.

Cow differences again were determined, and the results are given in Table 10.

TABLE 10
Statistical summary of the effect of the form of alfalfa on individual cow differences between that fed and refused

Chemical Constituent	Form of alfalfa		
	Baled	Chopped	Pelleted
Crude protein	NS [a]	NS	NS
Ether extract	$P < 0.01$	$P < 0.01$	NS
Crude fiber	NS	$P < 0.01$	NS
N.F.E.	$P < 0.01$	NS	$P < 0.01$
Ash	$P < 0.01$	$P < 0.01$	$P < 0.01$

[a] Nonsignificant

These results as well as those of Experiment I, based upon chemical analyses of roughage fed and refused, appeared to suggest the need for individual cow samples for chemical analysis when feeding either field-baled or chopped hays but not for the ground or pelleted. The results further indicated that the cows sorted out and consumed the leaves from the baled hay and the stems from the dehydrated chopped hay. Just why this differential sorting took place was not known.

Live weight. The average live weights of the 18 cows were 1,000, 1,007, and 962 lb. for the baled, chopped, and pelleted forms of alfalfa, respectively. The average weight of all cows during Period I was 989 lb.; during Period II, 988 lb.; and 992 lb. in Period III. As the foregoing data indicated, ration effects were significant ($P < 0.01$), whereas the period did not appreciably affect live weights.

SUMMARY

In two experiments, the relative value for milk production of various forms of alfalfa fed as the sole source of roughage was determined. In the first experiment, in which comparisons between field-cured and field-baled, artificially dried-ground and artificially dried-pelleted hays were made, actual milk production was greater and percentage of fat lower during the feeding of the pelleted form. No appreciable differences between hays were observed when milk yields were equated to FCM. In the second experiment, in which comparisons between field-cured and field-baled, artificially dried-chopped and artificially dried-pelleted hays were made, lowered hay consumption accompanied by lowered percentages of fat and FCM were observed during the feeding of the pelleted form. This was attributed to the hardness of the pellets.

Chemical analyses of the hays fed and refused indicated considerable cow sorting of the field-cured and field-baled and artificially dried-chopped hays. Cows also were observed to differ with respect to this sorting.

ACKNOWLEDGMENTS

The authors are grateful to G. R. Farrington, R. D. Mochrie, and G. Smith for technical assistance during the course of this experiment, to Geoffrey Beall for aid in the statistical treatment, and to H. J. Fisher, Connecticut Agricultural Experiment Station, for the proximate analyses of the feeds.

REFERENCES

(1) Balch, C. C. Factors Affecting the Utilization of Food by Dairy Cows. 1. The Rate of Passage of Food through the Digestive Tract. British J. Nutrition, **4**: 361-368. 1950.

(2) Bechdel, S. I., and Williams, P. S. The Pennsylvania Agricultural Experiment Station 42nd Annual Report. Pa. Agr. Expt. Sta. Bull. 243. 1929.

(3) Blosser, T. H., Murdock, F. R., Lintott, R. E., Erb, R. E., and Shaw, A. O. The Use of Dehydrated Forages in Dairy Cattle Rations. II. Comparative Values of Finely Ground, Chopped and Pelleted Dehydrated Alfalfa as Grain Replacements for Lactating Cows. J. Dairy Sci., **35**: 515-523. 1952.

(4) Cochran, W. G., Autrey, K. M., and Cannon, C. Y. A Double Change-Over Design for Dairy Cattle Feeding Experiments. J. Dairy Sci., **24**: 937-951. 1941.

(5) DRAKELEY, T. J., AND WHITE, M. K. The Joint Influence of the Period of Lactation and the Age of the Cow on the Yield and Quality of the Milk. J. Agr. Sci., **18**: 496-506. 1928.

(6) ESPE, D. *Secretion of Milk.* 3rd ed. The Iowa State College Press, Ames. 1948.

(7) ESPE, D., AND CANNON, C. Y. The Influence of the Physical Character of the Roughage on the Per Cent of Fat Secreted in the Milk. J. Dairy Sci., **22**: 799-801. 1939.

(8) GAINES, W. L. Milk Energy Yield and the Correlation Between Fat Percentage and Milk Yield. J. Dairy Sci., **23**: 337-342. 1940.

(9) HART, E. B., KLINE, O. L., AND HUMPHREY, G. C. The Effect of Artificial Drying on the Nutrients of Alfalfa Hay. J. Agr. Research, **45**: 507-511. 1932.

(10) HOPE, E. B., ERB, R. E., BLOSSER, T. H., ASHWORTH, U. S., AND SHAW, A. O. The Use of Dehydrated Forages in Dairy Cattle Rations. I. Grain Substitution with Finely Ground Material. J. Dairy Sci., **33**: 306-314. 1950.

(11) HUFFMAN, C. F. Roughage Quality and Quantity in the Dairy Ration. J. Dairy Sci., **22**: 889-980. 1939.

(12) KNOTT, J. C., AND HODGSON, R. E. The Feeding Value of Artificially Dried Pasture Herbage for Milk Production. J. Dairy Sci., **17**: 409-416. 1934.

(13) MORRISON, F. B. *Feeds and Feeding.* 21st ed. The Morrison Pub. Co., Ithaca, N. Y. 1948.

(14) MORROW, K. S., AND LAMASTER, J. P. Ground Hay for Milk Production. S. C. Agr. Expt. Sta. Bull. 255. 1929.

(15) NEWLANDER, J. A. The Feeding Value of Artificially Dried Grass. Vt. Agr. Expt. Sta. Bull. 350. 1933.

(16) NEWLANDER, J. A., ELLENBERGER, H. B., CAMBURN, O. M., AND JONES, C. H. Digestibility of Alfalfa, Timothy and Soybeans as Silages and as Hays. Vt. Agr. Expt. Sta. Bull. 430. 1938.

(17) PATUREL, G. Cited by C. F. HUFFMAN. Roughage Quality and Quantity in the Dairy Ration. J. Dairy Sci., **22**: 906. 1939.

(18) POWELL, E. B. One Cause of Fat Variation in Milk. Proc. Am. Soc. Animal Production, p. 40. 1938.

(19) RAGSDALE, A. C., AND TURNER, C. W. The Stage of Lactation as a Factor in the Variation of the Percentage of Fat in Cow's Milk. J. Dairy Sci., **5**: 22-26. 1922.

(20) RUPEL, J. W., ROCHE, B. H., BOHSTEDT, G., AND FULLER, J. G. Chopping Hay for Livestock and Steaming and Predigesting Feeds. Wis. Agr. Expt. Sta. Research Bull. 102. 1930.

(21) SNEDECOR, G. W. *Statistical Methods.* 3rd ed. The Iowa State College Press, Ames. 1940.

(22) TYZNIK, W., AND ALLEN, N. N. The Relation of Roughage Intake to the Fat Content of the Milk and the Level of Fatty Acids in the Rumen. J. Dairy Sci., **34**: 493. 1951.

(23) WATSON, S. J. Investigations into the Intensive System of Grassland Management. IV. The Digestibility and Feeding Value of Artificially Dried Grass. J. Agr. Sci., **21**: 414-422. 1931.

(24) WATSON, S. J., AND FERGUSON, W. S. The Nutritive Value of Artificially Dried Grass and its Effect on the Quality of Milk Produced by Cows of the Main Dairy Breeds. J. Agr. Sci., **20**: 189-209. 1936.

(25) WATSON, S. J., FERGUSON, W. S., AND HORTON, E. A. The Time of Cutting Hay, and the Losses Entailed During Haymaking. J. Agr. Sci., **27**: 224-256. 1937.

PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

LORE A. ROGERS obtained his B.S. degree in Agriculture in 1896 from the Univ. of Maine. He continued his studies in the graduate school of the Univ. of Wisconsin. From 1899 to 1902 he was Assistant in Bacteriology at the Geneva Experiment Station. For the next 40 years he served as chief of the Research Laboratories of the Bureau of Dairy Industry, from which he retired in 1942.

Among the honors bestowed upon L. A. Rogers were the Borden Award and a Doctor of Science degree from both the Univ. of Maryland and the Univ. of Maine. He is a past president of the Society of American Bacteriologists and was an associate editor of their journal. He also has been associate editor of the Journal of Dairy Science and Biological Abstracts. He was an official delegate to four World Dairy Congresses.

Dr. Rogers has published extensively in the field of dairy bacteriology, his principal contribution being the taxonomy of various groups of organisms, studies of the factors controlling the rate of multiplication of bacteria, and the limits of population in bacterial cultures. His scientific articles and bulletins total 86.

In 1928 the associates of Dr. Rogers published the book, "Fundamentals of Dairy Science," which was dedicated to the man who had contributed much to the fundamental as well as applied fields of dairy bacteriology.

Often when a professional man retires, his active life ceases, but not in the case of L. A. Rogers. A recent letter to the editor from DR. J. M. SHERMAN of Cornell states, "Upon retirement Dr. Rogers went back to his native Patten, Maine, which is up in the potato and lumbering country. He has built a very modern house with all sorts of automatic conveniences and gadgets. He also has a wonderful camp on a lake near Patten that he has been developing and enlarging for more than 40 years.

"As you perhaps know, he started a creamery at Patten some years ago, and as a result there is now a lot of milk produced up in the potato country where there was little produced before. This business, the Katahdin Creamery, has grown and thrived to such an extent that he now has another milk plant in Caribou, Maine, some 75 miles away in the northeastern part of the state, and the whole business has become so large and prosperous that his son, John, a Harvard Law School graduate, has entirely given up his successful law practice to take over the management of the creamery company. Dr. Rogers makes all the bacterial counts and runs other quality control tests of both plants. That gives him part time employment, which he seems to enjoy very much.

"He also has all sorts of other interests and activities that occupy his time and, as anyone would expect who knows Dr. Rogers, he is always busy making something in his complete shop in the basement of his home. In addition there is always any amount of work to be done at his camp, which he especially loves, and where he spends as many summer months as possible. A recent activity was to build, entirely with his own hands, a bateau beginning with the selection and cropping down of a 'stick' of white pine 34 inches in diameter, from which to get the necessary lumber of suitable length. The resulting boat is not only a work of art and fine craftmanship, but entirely seaworthy and a means of transportation of great utility and remarkable performance."

February 7 next, L. A. Rogers will be 79 years old. Here is a man who has made a remarkable success as a scientist and then in retirement has added to his fame by building for himself another enviable record as a successful businessman, an achievement of no little distinction in itself. The dairy industry is proud to have him as one of its pioneers. The fullness of his life and the glory of his accomplishments should serve as an inspiration to today's crop of budding dairy scientists. It is men like L. A. Rogers who make this a better world in which to live.

Changes in Staff Members

J. D. DONKER has joined the department at the Univ. of Georgia to direct work in dairy cattle nutrition. Dr. Donker went to Georgia from Minnesota, where he received his doctor's degree in 1952. W. J. MILLER also will do work in dairy cattle nutrition at Georgia. He is a native of North Carolina but took his degree at Wisconsin. During the past year he has been at the Univ. of Illinois.

CARL W. VAN DER ZANT has joined the A. & M. College faculty at Texas as Assistant Professor in Dairy Manufactures. A native of Holland, he entered Iowa State College in 1949 and obtained his Ph.D. degree in 1953.

WILLIAM R. THOMAS has been appointed Instructor in Dairy Manufacturing at the Univ. of Wyoming. He will replace DR. H. S. WILLARD during his leave with the Point Four pro-

gram in Jordan. Mr. Thomas, a 1951 graduate of Oklahoma, obtained his M.S. degree from Ohio State in 1952. For the past two years he has been a member of the staff of the Evaporated Milk Association.

D. M. GRAHAM has been appointed Associate in Dairying at the South Carolina Agricultural Experiment Station. He received his B.S. degree from Mississippi State College and his M.S. from Iowa State. He has completed work for his Ph.D. at Ames. Mr. Graham will have charge of the reactivated blue cheese project and will do research work on other dairy products.

Another new staff member at Clemson is JAMES T. LAZAR, JR., who will have charge of the Dairy Department processing plant, will conduct dairy products research, and will teach introductory dairying. Mr. Lazar, native of South Carolina, graduated from Clemson in 1943. After being discharged from the Navy in 1946 he joined the staff at Clemson. In 1948 he went to Cornell for his M.S. degree. After two more years at Clemson he resigned to enter North Carolina State College, where he has completed all requirements for a Ph.D. degree.

GEORGE R. W. BENTLEY, JR., a graduate of the Univ. of Georgia, has joined the staff of Clemson College. He is to be in charge of operations on the Clemson Dairy Farm.

ROGER A. MORRISON, formerly of the staff of Washington State College, has accepted an appointment as Extension Dairyman at the Ohio State University to fill a position left by the resignation of RALPH PORTERFIELD.

EARL VOELLER of the staff of the Ohio Agricultural Experiment Station, who for several years has been identified with Ohio's federal breeding project, has resigned to take over an extensive Ayrshire breeding enterprise and farm of his own. HERMAN RICKARD has been appointed in his place.

CURTIS W. RICHARDSON has accepted an appointment at Eastern Oklahoma A. & M. College, Wilburton, Okla.

EUGENE J. FINNEGAN has been appointed Associate Professor of Dairy Manufacturing at Virginia Polytechnic Institute. He will fill the position left vacant by the death of DR. C. C. FLORA. Professor Finnegan was a member of the staff of the Department of Dairy Industry, Univ. of Massachusetts, where he received both undergraduate and graduate degrees.

N. R. THOMPSON has returned to the Department of Dairy Husbandry, Virginia Polytechnic Institute, after an educational leave of three years. During that time he studied at North Carolina State College and Michigan State College.

Completed Theses

M.S. Degree:

CURTIS W. RICHARDSON—The effect of aureomycin upon the growth of dairy calves when administered orally, subcutaneously, and extramuscularly. Oklahoma A. and M. College.

GEORGE R. CLAWSON—Alfalfa vs. prairie hay from birth or after eight weeks of age for young dairy calves with or without aureomycin supplement. Oklahoma A. and M. College.

JACK LEONARD BARRS—Detecting butterfat adulteration by Reichert-Meissl number changes on fractions selectively solidified. Univ. of Florida.

PETER W. FINKEL—The errors associated with estimations of the reproductive efficiency of bulls on nonreturn data. Virginia Polytechnic Institute.

Dr. Trebler Retires

H. A. TREBLER, Director of engineering research and development of National Dairy Research Laboratories, Inc., Oakdale, Long Island, has been granted early retirement as of August 1, 1953. Dr. Trebler is planning to devote a portion of his time to consulting work in the field of dairy engineering.

Dr. Trebler received his education in Norway and Switzerland, worked for some time in France, came to this country in 1924, and has been with the research laboratories of National Dairy since 1930. He has a large number of papers and patents to his credit and has been active in many fields of dairy engineering. As a member of the Plant Committee of the Milk Industry Foundation he contributed a great deal to the Plant Manual issued by that Committee. As Chairman of the Sanitation Engineers Committee of the Pennsylvania Assoc. of Milk Dealers, he was largely responsible for the issuance of their "Dairy Waste-Saving and Treatment Guide for Milk Plant Operators," which has received wide distribution.

Professor Loosli to Spend Year in Philippines

Professor and MRS. J. K. LOOSLI and their three children left on June 20 for the Philippines, where they joined other members of the Cornell staff in the cooperative Mutual Security Aid program with the College of Agriculture at Los Banos. Enroute to the Philippines they made short stops in Hawaii, Japan, and Hong Kong.

Professor Loosli will assist in the development of teaching and research in animal husbandry. After a year in the Philippines, the Looslis will return through southern Asia and Europe, thus completing a circuit of the world.

Be Yourself

A Guest Editorial

Be it on the teaching staff, in the classroom, in the office or in the plant, the attitude of personnel is mainly responsible for the results obtained. It determines to a large degree the quality of teaching, the quality of the gradu-

ating student, the quality of our products, and the efficiency of our operations. The ever increasing complexity of our business, changes in our standards of living, meaning both work and leisure, the patronizing attitude of our government, the military situation, and the ever increasing demands of labor make the training and handling of personnel our number one problem in this mid-twentieth century.

H. F. Judkins

It is probably unwise for any class of workers, and that includes all of us, to be too intimate socially. If we know too much about each other's business, petty differences and jealousies are apt to arise and the grapevine flourishes. That is not to say, however, that we should be standoffish in our daily business relations. My observations are that there is still too wide a gap between management and the employee and possibly in some cases between the teacher and the student. I am sure in my teaching days the latter was the case.

How do we in the top echelons get that way?

Well, for one thing, we get so busy with the many "extra curricula" activities and doing the things we think we have to do that we do not take time to think about the things we ought to do.

Secondly, it seems to be difficult for some teachers and some executives to sit down occasionally with the student or the employee and help him analyze himself and perhaps offer suggestions that will make a better student or a better employee. Nothing can be more important.

In the third place, there is the individual who takes himself too seriously. It is beneath his dignity to say anything more than a real stiff, unsmiling "good morning" to Joe Dokes if in fact he says anything at all.

The answer to good relations between each other and having good teamwork lies in acting toward others as you would have them act toward you. That means acting natural. In other words, Be Yourself. Both you and your associates will feel better.

H. F. Judkins,
National Dairy Products Company, Inc.

H. A. Ruehe Dies

H. A. Ruehe, Professor of Dairy Science at the Univ. of Illinois, died suddenly October 8, at his home in Urbana. Dr. Ruehe was born in Gilman, Illinois, June 26, 1888. His B.S. and M.S. degrees were obtained from the Univ. of Illinois and his doctorate from Cornell Univ.

Dr. Ruehe joined the staff of the Univ. of Illinois in 1912 and served as head of the Department of Dairy Husbandry from 1921 to 1943. From 1943 to 1945 he was on leave to serve as secretary of the American Butter Institute. He was president of the A. D. S. A. in 1935-36 and was associate editor of the Journal of Dairy Science for a number of years. In recent years he had been active in cream quality control work in the state of Illinois. He is survived by his wife and two sons.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

766. Molybdenum poisoning of cattle in the Swan River Valley of Manitoba. H. M. CUNNINGHAM, J. M. BROWN, and A. E. EDIE, Univ. of Manitoba, Winnepeg, Man., and Cornell Univ., Ithaca, N. Y. Canad. J. Agr. Sci., **33**, 3: 254-260. 1953.

Toxic herbage, containing up to 25.6 p.p.m. of Mo. was found to be the cause of cattle poisoning in an area of western Manitoba. Symptoms include severe diarrhoea, emaciation, anaemia, fading of hair color, excessive craving for salt and often death. Some evidence showed that the toxicity may pass into the milk and affect the calf but that cattle soon develop a resistance to the passage of ingested Mo. into the milk. The disease was produced experimentally by drenching with ammonium molybdate but a higher intake was required. Toxicity was overcome by the daily administration of 2 g. of copper sulfate either as a drench or salt lick. Cobalt was found to be of no benefit.

O. R. Irvine

767. Dihydrostreptomycin dose-serum level relations in cattle. P. B. HAMMOND, Univ. of Minn., St. Paul. J. Am. Vet. Med. Assoc., 122, 203-206. 1953. (Chem. Abstr., **47**: 8241d. 1953.)

S. Patton

BOOK REVIEWS

768. Sanitary Milk Control and its relation to the sanitary, nutritive and other qualities of milk. A. C. DAHLBERG, H. S. ADAMS, and M. E. HELD. Publication 250, National Research Council, Washington, D. C. 1953.

Birmingham, Boston, Houston, Louisville, Minneapolis, Rochester (N. Y.), Sacramento and Washington (D. C.) were the cities chosen as representative, by the criteria employed, for this survey. Producing farms and milk plants studied were chosen by definite procedures which helped to assure that all representative types were covered in the survey. The publication should be consulted for the details of selection procedures and examination techniques. The findings are interpreted as showing the need for only a limited number of basic requirements to insure a wholesome milk supply. Many of the items now found in milk ordinances, particularly relative to producing farms, should be recommendations, rather than requirements. Healthy cows, a safe water supply, sanitary disposal of human excreta, healthy personnel, and adequate fly control are important in preventing contamination of milk with pathogenic bacteria. Wash vats and hot water in milk houses were essential for clean dairy utensils. Cleaning of the udders before milking resulted in raw milk of low coliform count, the latter being an indication of cleanliness. Cooling of all milk to 50° F. or below, except that which is to be delivered to the processing plant immediately after milking, is essential for marketing of low-count milk. Routine farm inspections at least twice yearly are recommended. Semi-monthly tests should include temperature, sediment, "total bacterial count," and thermoduric count, the last especially when the total bacterial count was not reduced sufficiently by pasteurization. A total bacterial plate count of 200,000 per ml. for raw milk is suggested as a standard because it can be attained readily with reasonable facilities and good methods of production; however, 51% of the samples studied exceeded this standard.

All pasteurized milk examined was "safe and wholesome and generally complied with all standards." This prevented evaluation of the worth of individual regulatory requirements. Temperature, sediment, flavor, "total bacterial count," coliform count and phosphatase tests are suggested in addition to inspections of plants at least once a week. Some samples of pasteurized milk showed poor keeping quality at 44° F.; therefore, keeping quality tests should be made once a week for flavor and for psychrophilic and coliform bacteria. "Feed" flavors were most common in freshly pasteurized milk, while "unclean" and "oxidized" were the predominant off-flavors after storage. Sale of only one grade of milk, "Grade A Pasteurized" or equivalent, was recommended, with provisions also being made for "Certified Pasteurized Milk."

"The fresh pasteurized milk showed slightly less solids-not-fat, total protein and ascorbic acid than generally given in the literature, and slightly more thiamine." The freezing point of milk as examined in six of the cities "was significantly above the standard for pure milk

established by the Association of Official Agricultural Chemists, as shown by statistical study." This publication is recommended reading for all who are concerned with the milk industry. F. E. Nelson

BUTTER

769. Undersoegelser vedroerende smoer og smoerfremstilling. F. 604. Forsoeg med raps- and soyaolie til malkekvaegets foder (Research in butter and buttermaking. F. 604. Addition of rapeseed and soybean oil to feed for dairy cows). A. N. Fisker and H. Rambak, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull. 80: 71-87. 1953.

Rapeseed oil or soybean oil was added to the regular feed for dairy cows in an attempt to overcome the "short" and "crumbly" defects which occurred in butter, especially during the winter months.

Additions of 1.5-3% rapeseed oil or 2-5% soybean oil, depending on the original diet, increased the iodine no. of the butter from about 26 to about 30, which was sufficient to overcome the defects. After 3 mo. in cold storage no increase in degree of oxidation (organoleptic) or in peroxide no. could be detected due to the added oils. Acid no., pH and bacteriological quality also were similar to that of the control butter. T. Kristoffersen

770. Undersoegelser vedroerende smoer og smoerfremstilling. F. 626. Tankkoeling kontra afkoeling i pladekoeler (Research in butter and buttermaking. F. 626. Tank cooling versus cooling in platecooler). Aa. H. Pedersen and A. N. Fisker, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull. 80: 87-91. 1953.

No significant difference in fat loss or in the consistency of the butter could be observed when ripened cream was cooled prior to churning in the ripening tank or by pumping it through a platecooler. T. Kristoffersen

771. Continuous butter-making process and apparatus. W. Wilsmann (assignor to Westfalia Separator, A. G.). U. S. Patent 2,649,377. 9 claims. Aug. 18, 1953. Official Gaz. U. S. Pat. Office, 673, 3: 789. 1953.

Cream is churned into butter granules and buttermilk in a chamber with rotating, agitating blades. The butter granules and buttermilk are delivered into the lower end of a slanted cylinder, in which an augur-like worm gear rotates in the direction to force the butter granules upward. The buttermilk escapes through a strainer in the bottom of the cylinder. The butter granules travel upward, propelled by the worm, where they are squeezed, mixed with salt if desired, and worked to form butter, which is then extruded continuously through a tapered orifice at the top end of the cylinder. R. Whitaker

772. Device for making butter pats. M. J. Weinberg. U. S. Patent 2,645,852. 17 claims. July 21, 1953. Official Gaz. U. S. Pat. Office, 672, 3: 685. 1953.

An apparatus for cutting individual servings of butter from prints. R. Whitaker

773. Kinetics of butter formation. A. Grishchenko, Refrig. and Dairy Inst., Leningrad. Molochnaya Prom., 14, 4: 28-32. 1953. (Chem. Abstr., 47: 7688e. 1953.) S. Patton

774. Technology of Vologda (region) butter. A. Zheltakov and V. Shershneva. Molochnaya Prom., 14, 5: 12-18. 1953. (Chem. Abstr., 47: 7688g. 1953.) S. Patton

775. Quality, stability, and peculiarities of butter prepared by the Meleshin method. A. Titov. Molochnaya Prom., 14, 4: 13-16. 1953. (Chem. Abstr., 47: 7688h. 1953.) S. Patton

776. Oxidation number of fat and butter. Ya. Zaikovskii and A. Chizhkova. Molochnaya Prom., 14, 4: 32-33. 1953. (Chem. Abstr., 47: 7689i. 1953.) S. Patton

777. Use of yeast culture in making of butter. G. Blok and S. Geimbert, Dairy Inst., Vologda. Molochnaya Prom., 14, 6: 17-21. 1953. (Chem. Abstr., 47: 8278f. 1953.) S. Patton

778. Durable butter. Frederik D. Tollenaar (to Nederlandsche Organisatie voor Toegepast-Natuurwetenschappelijk Onderzoek ten behoeve van de Voeding). U. S. 2, 638, 420, May 12, 1953. (Chem. Abstr., 47: 8287a. 1953.) S. Patton

779. Moisture-meters used in the butter and oil industry. V. I. Polstyanoi. Maslobaino Zhirovaya Prom., 2: 18-20. 1953. (Chem. Abstr., 47: 6710g. 1953.) S. Patton

CHEESE

780. Fruity Flavor in Cheddar cheese. E. G. Hood, C. A. Gibson, and K. N. Smith, Canada Dept. of Agr., Ottawa. Sci. Agr., 32, 12: 638-644. 1952.

The effect of yeasts in producing fruity and other defective flavors in raw milk cheese was studied by examining 31 yeast cultures isolated from degraded fruity cheese containing high yeast counts. When grown in skimmilk only 3 produced a characteristic fruity flavor. These 3 cultures as well as cultures of *Alcaligenes viscosus, Escherichia coli* var. *neapolitana* and a *Micrococcocus* species were further examined by inoculating them into 240-lb. lots of raw milk and incubated at 70° F. for 7 hrs. before

being made into cheese. All of the 19 yeast inoculated vats were second grade at first grading because of sl. fruity, fruity or unclean flavors. The 10 vats inoculated with ropy milk bacteria were, in most cases, classed as sl. unclean. Milk with high yeast counts at rennetting produced cheese with strong fruity flavors.
O. R. Irvine

781. A short-time method of Cheddar cheese manufacture. H. E. WALTER, A. M. SADLER, J. P. MALKAMES JR., and C. D. MITCHELL, Div. Dairy Prod. Research, B.D.I. Washington, D.C. Am. Dairy Prod. Rev., **15**, 8: 6. 1953.

The new B.D.I. method for cheese manufacture requires only 2½ hrs. from the time rennet is added until the curd is pressed, in contrast to the 5½ to 6½ hrs. of present methods. In the process 2 starters are used. One is the usual type and the other is a non-hemolytic strain of *S. durans* which is heat and salt tolerant. Milk is ripened and set at 88° F. The curd is cut and cooked to 100° F. in a regular cheese vat. Curd with whey is then pumped into a revolving, perforated drum which removes about ½ the whey. The partly drained curd falls into a cooking kettle where it is heated to between 115 and 120° F. and then salted. The salted curd and whey is pumped into a specially devised cheese hoop in a hooping tank containing whey at between 115 and 120° F. After excess whey has drained, the cheese is pressed and dressed. Conventional packaging methods are used. Cheese cured at 55° F. for about three months has a mild flavor that appeals to consumers. Details of special equipment are given. T. J. Claydon

782. Cottage cheese manufacture using concentrated dairy products. H. H. WILKOWSKE and L. E. MULL, Fla. Agr. Expt. Sta., Gainesville. Ann. Rept. 79. 1952.

Use of reconstituted skimmilk of 13% solids resulted in satisfactory cottage cheese, but when the solids were below 11%, the cheese was unsatisfactory. Cutting at 0.53% titratable acidity (recommended for fresh skimmilk cottage cheese) was not satisfactory for the reconstituted product containing 13% SNF.
R. W. Hunt

783. The effect of sodium hexametaphosphate on flavor and texture of frozen cottage cheese. E. MEDVED, I. PRUDENT, and L. H. BURGWALD, School of Home Econ. and Dept. of Dairy Technol., Ohio State Univ., Columbus. Food Technol., **7**, 8: 316-318. 1953.

Sodium hexametaphosphate (Calgon) was added to pasteurized skimmilk in concentrations of 0.00, 0.12 and 0.36% and the milk made into cottage cheese by the short-time method. The fresh curd was placed in waxed pint containers and frozen at −10° F., and then transferred to a freezing cabinet at 0° F. The addition of Calgon to the milk had no beneficial effect on the texture and flavor of the thawed cottage cheese after 1, 4, and 8 wks. of frozen storage. In the amounts used, Calgon had no appreciable effect on the moisture content or the cream absorbing property of the cottage cheese curd. E. R. Garrison

784. Theorie zur Erklärung des Haftvermögens der Käsebruchkörner. (A theory explaining the adhesive power of curd particles in cheese.) (English summary.) M. E. SCHULZ. Die Milchwissenschaft. **8**, 3: 78-79. 1953.

Cooking curd particles alters their behavior to each other. The adhesive power of the Emmenthaler cheese curd particles is at its maximum at cooking temperatures of from 40-45° C. At 55° C. the curd particles fail to adhere to each other and it is only by means of outside applied pressure that the curd particles will stay together.

It is postulated that during the cooking process the particles shrink and expel moisture. Factors causing cessation of shrinkage and inhibition of moisture by the curd particles will display the act of suction by the particles upon its immediate surroundings and thus bring about the adhesion of curd particles. Protein breakdown, decreasing temperatures, and expansion of curd particles in the presence of salts aid the adhesion of particles.
I. Peters

785. Protectively coated edible cheese morsels and method of producing same. J. KRUSI. U. S. Patent 2,648,607. 6 claims. Aug. 11, 1953. Official Gaz. U. S. Pat. Office, 673, 2: 520. 1953.

Small pieces of cheese are coated with a layer of whey solids to impart brittleness and to protect the particles from mold and the formation of a rancid flavor. R. Whitaker

786. Cheese slicing utensil. H. L. NELSON. U. S. Patent 2,649,658. 2 claims. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 929. 1953.

A device for holding a block of cheese in position for slicing by hand. R. Whitaker

787. Mixing kettle for cheese curd. E. ZEILER and K. LENZ (assignors to Anton Steinecker Maschinenfabrik A. G.). U. S. Patent 2,650,079. 1 claim. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 1050. 1953.

The important feature of this invention is the type of agitator used to stir melted or semiliquid curd as it is mixed to form various types of cheese foods. Attached to a vertical shaft extending upward through a tube in the center of the kettle are a series of arms, to which are attached pivoted blades which oscillate to and fro in a limited manner as the shaft is reciprocately rotated. R. Whitaker

788. Report on preparation of sample of creamed cottage cheese with the Waring blendor. S. H. PERLMUTTER, Food and Drug Adm., Federal Security Agency, Minneapolis, Minn. J. Assoc. Off. Agr. Chemists, **36**, 2: 187-190. 1953.

A sample of creamed cottage cheese (339.1 g.) having an initial temperature of 16.5° C. lost 0.1 g. of weight after mixing in a Waring blendor for 1.5 min. During this time the temp. increased to 19.5° C. Although the sample increased to 25.5° C. after mixing for 7.5 min., the weight loss was still 0.1 g. After mixing for 19.5 min. the temp. increased to 44.5° C. and the weight loss amounted to 0.8 g.

The method recommended for preparing samples of creamed cottage cheese was to place 200-600 g. sample at 15° C. in a quart cup of a high speed blendor and blend for 2 to 5 min. Final temp. should not exceed 25° C.

F. J. Babel

789. The sampling of Cheddar cheese for routine analyses. W. V. PRICE, W. C. WINDER, A. M. SWANSON, and H. H. SOMMER, Univ. of Wis. J. Assoc. Off. Agr. Chemists, **36**, 2: 524-538. 1953.

Analyses indicated that the sample preferred for commercial analysis of a Cheddar is obtained by drawing 2 plugs, one from the top, the other from the bottom of the cheese. Each plug extends perpendicularly halfway through the cheese and is drawn from the center of each flat surface. One in. of the rind end of each plug is used to close the orifice of the trier hole. Plugs are $\frac{5}{8}$ to $\frac{3}{4}$ in. in diameter. Under satisfactory commercial conditions, analyses of such a sample from a single Cheddar cheese estimated the mean of its vat lot within control limits of $\pm$ 0.759. F. J. Babel

790. The determination of moisture in process American cheese and process cheese food. J. H. COOK, H. C. FOLLSTAD, and W. W. FISHER, Kraft Foods Co., Glenview, Ill. J. Assoc. Off. Agr. Chemists, **36**, 1: 132-137. 1953.

The official method for determination of moisture in cheese (100° C. vacuum oven) gave a constant weight of process American cheese after about 4 hrs. With process cheese food the sample continued to lose weight after the moisture was removed and in many cases gave a dark brown color to the residue. When process cheese food was spread thinly over the bottom of a moisture dish, the residue was much lighter and the sample did not continue to lose weight. A glass rod technic was developed in which small "mashers" (glass rods with flat ends about 1.5 in. long) were tared with the moisture dish. A single lump of sample was weighed and the masher used to spread the sample in a thin layer over the bottom of the dish.

Moisture determinations on 108 samples of process cheese food indicated that the official method gave results which averaged 0.3% higher than the glass rod method. Moisture analyses on 105 samples of process American cheese showed that the glass rod method gave values slightly higher than the official method but 85% of the samples showed less than 0.2% variation. It appeared that if moisture was removed rapidly from process cheese food over the first hour, less burning resulted. Smearing the sample over the dish was conducive to this condition. F. J. Babel

791. The cause of the darkening of foil (in processed cheese). I. ALYAMOVSKII, Process Cheese Plant, Leningrad. Molochnaya Prom., **14**, 5: 26. 1953. (Chem. Abstr., **47**: 7685b. 1953.) S. Patton

792. Prevention of mold development of cheese rind. H. HENDRICKX and A. DE VLEESCHAUWER, Rijkszuivelsta. Ghent, Belg. Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Ghent, 17: 669-677. 1952. (Chem. Abstr. **47**: 7690b. 1953.) S. Patton

793. Modifications of fats formed during the ripening of different types of cheeses. M. BEJAMBES, S. SAVOIE, and S. CLUZEL, Milk Research Sta., Paris. Ann. inst. natl. recherche agron., Sér. E, Ann. technol. agr., **1**, 1: 23-30. 1952. (Chem. Abstr., **47**: 8277h. 1953.) S. Patton

794. Cheddar-cheese flavor and its relation to tyramine production by lactic acid bacteria. J. C. DACRE, Dairy Research Inst., Palmerston North, N. Z. J. Dairy Research, 20, 217-223. 1953. (Chem. Abstr., **47**: 8277i. 1953.) S. Patton

795. Soft surface-cured cheese. JOHN H. KRAFT (to Kraft Foods Co.). U. S. 2,641,545, June 9, 1953. (Chem. Abstr., **47**: 8287e. 1953. S. Patton

CONDENSED AND DRIED MILKS; BY-PRODUCTS

796. Increasing the food value of whey by yeast fermentation. I. Preliminary studies on the suitability of various yeasts. V. E. GRAHAM, D. L. GIBSON, H. W. KLEMMER and J. M. NAYLOR, Univ. of Sask., Saskatoon. Canad. Jour. Technol., **31**, 4 and 5: 85-91. 1953.

The following cultures were studied as to their suitability for increasing the food value of whey: *Candida krusei*, *Torula utilis*, *Torula utilis thermophilus*, and *Torula cremoris*. The latter species was the only one able to utilize lactose and appears most suitable for commercial consideration. Highest cell counts were obtained when a 5-10% inoculum was used and the medium aerated after the first 24 hrs. Sup-

plementary nitrogen in the form of ammonium salts stimulated growth. The optimum pH and temperature requirements for the maximum production of cells in 72 hrs. were 4.4-6.8 and 25-30° C. respectively. Pasteurized rennet whey was the preferred medium for *Torula cremoris* as compared to sterilized rennet or sterilized acid wheys. O. R. Irvine

797. Increasing the food value of whey by yeast fermentation. II. Investigations with small scale laboratory fermenters. V. E. GRAHAM, D. L. GIBSON, and H. W. KLEMMER, Univ. of Sask., Saskatoon. Canad. J. Technol., **31**, 4 and 5: 92-97. 1953.

Two small-scale laboratory fermenters, of 2 and 8 l. capacity respectively, are described for use in the rapid fermentation of whey by *Torula cremoris*. Growth occurred more rapidly in the larger fermenter, apparently because of better aeration. In 24 hrs. lactose was reduced to 0.1% and protein raised from 0.89 to 1.06% in a typical run. Riboflavin was also increased. Whey diluted to a lower lactose concentration allowed slightly more efficient utilization during the first 24 hrs. while whey concentrated 2 to 1 provided high final yeast yields but less efficient lactose conversion. Under conditions of poor aeration, alcohol production was high and yeast cell production low whereas the ratio was reversed under efficient aeration. O. R. Irvine

798. Increasing the food value of whey by yeast fermentation. III. Pilot plant studies. V. E. GRAHAM, D. L. GIBSON, and W. C. LAWTON. Univ. of Sask., Saskatoon. Canad. J. Technol., **31**, 4 and 5: 109-113. 1953.

Two methods are described in which pilot plant sized lots of pasteurized cheese whey were fermented by *Torula cremoris*. One made use of a vacuum pan and another a metal barrel. The fermentation product was sufficiently depleted in lactose to permit drying on an atmospheric drum dryer. A non-caking, non-hygroscopic powder was produced containing approximately 39% protein, 19% fat, 30% ash and 12% nitrogen-free extract, on a moisture-free basis. O. R. Irvine

799. Report on milk by-products in mixed feeds. Total solids determination. A. O. CALL, Western Condensing Co., Appleton, Wis. J. Assoc. Off. Agr. Chemists, **36**, 2: 213-219. 1953.

Three commercial samples of condensed milk by-product feeds were submitted to 16 collaborators for total solids determinations in triplicate by the following methods: 1. A 10-15 min. preliminary heating on a steam bath followed by 3 hr. in an air oven at 98-100° C.; 2. Same as procedure 1, except an excess of zinc oxide was added to the dish to neutralize any acids present; 3. Same as procedure 1 except the sample was neutralized with sodium hydroxide prior to drying. Results obtained by method 1 were significantly lower than those obtained by methods 2 or 3, being only 94 to 98.7% as great. There was little difference in the replicability of any of the methods, but the ability of some collaborators to repeat themselves varied widely. F. J. Babel

800. Milk product and process. D. D. PEEBLES and M. D. GIRVIN (assignors to Western Condensing Co.). U. S. Patent 2,650,879. 2 claims. Sept. 1, 1953. Official Gaz. U. S. Pat. Office, 674, 1: 208. 1953.

Pasteurized concentrated skimmilk is mixed with a homogenized, sterilized emulsion of fat and sodium caseinate soln. and the mixture spray dried. The emulsion has the following composition: 45 to 89% water, 10 to 52% fat, and 1.2 to 3.1% sodium caseinate. The dried product has good fat stability. R. Whitaker

801. Milk product enhanced in amino acids and peptides. F. H. TINKLER, R. C. STRIBLEY, and J. G. KENNEDY (assignors to American Home Products Corp.). U. S. Patent 2,650,166. 4 claims. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 1075. 1953.

The proteins of whey are digested with a proteolytic enzyme to form soluble amino acids and peptides (non-precipitable by trichloroacetic acid). The digested whey is then treated first with a cation-exchange resin, then with an anion-exchange resin to reduce the ash content to not over 1% by weight. The pH is maintained between 4 and 7. R. Whitaker

802. The substances responsible for the specific odor of dry-milk products. S. SHTAL'BERG, and V. KRETOVICH. Molochnaya Prom., **14**, 5: 27-29. 1953. (Chem. Abstr., **47**: 7687b. 1953. S. Patton

803. Lactose crystallization in condensed milk by (the addition of) sugar. A. CHEBOTAREV, Milk Inst., Vologda. Molochnaya Prom., **10**, 11: 9-11. 1949. (Chem. Abstr., **47**: 7687e. 1953.) S. Patton

804. The determination of lime in milk. III. SIEGFRIED RAUSCHNING, Inst. Ernahrungs—u. Verpflegungswissenschaft, Potsdam-Rehbrueke, Ger. Pharm. Zentralhalle, **89**: 156-157. 1950. (Chem. Abstr., **47**: 7687h. 1953.) S. Patton

805. Milk product containing milk fat. CLAYTON A. KEMPF, JOY S. NEIDLINGER, AUBREY P. STEWART, JR., and PAUL F. SHARP (to Golden State Co., Ltd.). U. S. 2,638,418, May 12, 1953. (Chem. Abstr., **47**: 7697b. 1953.) S. Patton

806. Method for production of edible casein. P. F. D'YACHENKO, I. N. VLODAVETS, and E.

BOGOMOLOVA. Molochnaya Prom., **14**, 6: 33-36. 1953. (Chem. Abstr., **47**: 8277g. 1953.)
S. Patton

807. Powdered milk products for bread making. REGINOLD E. MEADE (to Western Condensing Co.). U. S. 2,602,746, July 8, 1952. (Chem. Abstr., **47**: 8286d. 1953.) S. Patton

808. Concentrated milk products. HERBERT E. OTTING and CECIL K. ORTMAN (to M & R Dietetic Laboratories, Inc.). U. S. 2,633,424, Mar. 31, 1953. (Chem. Abstr., **47**: 8286g. 1953.) S. Patton

809. Preserving condensed milk. ROBBERT DE VLETTER (to N. V. Lijempf). U. S. 2,641,549, June 9, 1953. (Chem. Abstr., **47**: 8287a. 1953.)
S. Patton

DAIRY BACTERIOLOGY

810. Manometric method for the evaluation of microbial activity of rumen with application to utilization of cellulose and hemicelluloses. R. H. MCBEE, Dept. of Bacteriol., Univ. of Mont. Appl. Microbiol., **1**, 2: 106-110. 1953.

A manometric method for detecting biochemical activity of the rumen microorganisms has been described. The fermentation of cellulose and hemicellulose was stimulated when cellulose was added to the hay diet of the sheep. The addition of hemicellulose to the feed increased the rate of hemicellulose fermentation but not that of cellulose. When the diet supplements were discontinued a decrease in these compounds was noted. H. H. Weiser

811. The effects of antibiotics on the bacterial plate counts of normal raw milk. E. I. STOLTZ and D. J. HANKINSON, R. R. Vanderbilt Co., Norwalk, Conn., and Univ. of Mass., Amherst. J. Milk and Food Technol., **16**, 4: 157-166. 1953.

The antibiotics used in mastitis therapy cause a marked reduction in the numbers of bacteria in raw milk. The concentration of antibiotics studied were: penicillin 1.0, 0.1 and 0.01 units per ml.; streptomycin 5.0, 1.0, and 0.1 mgm. per ml.; combination of penicillin and streptomycin respectively, penicillin 1.0 unit and streptomycin 5.0 mg. per ml., 0.1 unit and 1.0 mg., and 0.01 unit and 0.1 mg. Aureomycin was used in 0.5 megm, 0.25 megm, and 0.1 megm. per ml. concentrations.

All concentrations of antibiotics studied inhibited the growth of bacteria in raw milk stored for 48 hours at 7° C.

According to present bacteriological standards antibiotics in milk would render poor quality milk as an acceptable grade.
H. H. Weiser

812. Bacteriological evaluation of quaternary ammonium compounds alone and in detergent formulations. T. H. HUMPHREYS and C. K. JOHNS, Dept. of Agr., Ottawa, Canada. J. Milk and Food Technol., **16**, 4: 186-188. 1953.

Three quaternary detergent-sanitizers were compared by using a modified glass slide and Weber and Black methods at 5, 20 and 45° C. in artificial hard and distilled waters. To each detergent a constituent QAC was added according to recommended use-dilution of the homologous detergent sanitizer. Sample designated as A contained 50 p.p.m. of Hyamine 1622 as its germicidal component, samples B' and C contained 50 p.p.m. of B.T.C. and 200 p.p.m. of Roccal, respectively.

It appears that both methods gave comparable results regarding the effectiveness of detergent-sanitizers and their constituent QACs. However, the end point was reached more rapidly by the glass slide method. Both methods are satisfactory for evaluating QACs alone or in detergent formulations. H. H. Weiser

813. Ultraviolett-Luftentkeimung in einem Milchkühler-Raum. (Air sterilization by ultraviolet light in a milk-cooling room.) (English summary.) A. KOHN. Die Milchwissenschaft, **8**, 3: 90-92. 1953.

Low mercury vapor lamps that did not produce ozone were employed in experiments to determine the effectiveness of ultra-violet irradiation in a cold-storage milk room and on an open milk cooler.

Some reduction in bacterial counts was obtained after exposing air and surfaces to the rays of the lamps, however, the coli count was unchanged. Irradiation did not cause off flavors in the milk. I. Peters

814. Further studies on new milk-free media for the standard plate count of dairy products. R. BUCHBINDER, Y. BARIS, and L. GOLDSTEIN, N. Y. City Dept. of Health. Am. J. Pub. Health, **43**, 7: 869-872. 1953.

A comparison was made of the productivity of milk-free media and the present standard Difco tryptone glucose beef extract milk medium (T.G.E.M.). A milk-free dehydrated medium (Baltimore Biol. Lab.) containing 0.9% milk protein hydrolysate, 0.1% glucose and 1.5% agar was found to be an adequate replacement for T.G.E.M. medium. The results obtained on another milk-free medium (Difco) containing 0.5% tryptone, 0.1% glucose, 0.25% yeast extract and 1.5% agar also closely approximated the counts obtained with the T.G.E.M. medium. Increasing the amount of yeast extract from 0.25 to 0.35% increased the productivity of this latter medium.

The dehydrated yeast extract medium (Difco) was more productive than that prepared in the laboratory from Difco ingredients. The dehydrated medium also was more productive for pasteurized milk than for raw milk. The dehydrated T.G.E.M. medium was found to be more

productive than that prepared in the laboratory when plating pasteurized milk but the reverse was true for raw milk. Because of the differences in counts obtained with the laboratory prepared and the dehydrated media it is suggested that only the dehydrated form of Standard Methods media be used in order to obtain more uniform results in different milk control laboratories. D. D. Deane

815. Media used for microbiological assay of riboflavin using Lactobacillus casei. Method of assay and confidence limits attainable. M. F. CLARKE, Dept. of Chem., Med. Coll. of Va., Richmond. Analyt. Chem., **25**, 8: 1247-1252. 1953.

Changes in the media commonly employed to estimate riboflavin potency by microbiological assays were made to give results more susceptible to tests of reliability, and to allow simple means of calculation of confidence limits of estimated potency. The dose-response curves obtained using three unmodified media were compared to the results obtained when the media were modified as to glucose content, or glucose plus growth stimulants and buffer.
B. H. Webb

816. A new medium for the detection of enterococci in water. W. LITSKY, Dept. of Bact. and Pub. Health, Univ. of Mass., Amherst, W. L. MALLMANN and C. W. FIFIELD, Dept. of Bact. and Pub. Health, Mich. State Coll., East Lansing. Am. J. Pub. Health, **43**, 7: 873-879. 1953.

The authors believe that enterococci may be more useful than the coliform organisms as indicators of fecal pollution. A glucose-azide broth containing 2.0% tryptone, 1.5% dextrose, 0.50% NaCl, 0.27% K_2HPO_4, 2.27% KH_2PO_4 and 0.02% sodium azide was found satisfactory as a presumptive test for the presence of enterococci in sewage contaminated water. An ethyl violet-azide broth, which had the same composition except the sodium azide concentration was increased to 0.04% and in addition, 0.12 mgs. of ethyl violet was added per liter, was used as a confirmatory medium. D. D. Deane

817. Report of committee to confer with American Public Health Association on standard methods for the examination of dairy products. H. A. LEPPER, W. HORWITZ, and A. H. ROBERTSON. J. Assoc. Off. Agr. Chemists, **36**, 1: 46-47. 1953.

Among the noteworthy changes in the tenth edition of Standard Methods for the Examination of Dairy Products are the following: 1. A milk-free plating medium for the agar plate method will be substituted for the current tryptone glucose meat extract plating medium. 2. Systematic studies of 6 staining procedures have been completed; three procedures giving nearly identical results were an aniline oil-methylene blue stain and two acid- and water-free stains. 3. An all metal syringe will be recognized for measuring 0.01 ml. of milk. 4. Directions for the coliform test have been clarified. 5. Directions for the ring test for brucellosis are included. 6. Methods are outlined for sampling tanks and vats. 7. The use of a fine standard sediment mixture will supplement the use of the coarse standard sediment mixture. 8. A paragraph is included to govern gross misinterpretations on the activity of bacterial growth inhibitors. F. J. Babel

818. Bacterial enzymes. Relation between the gelatin liquefying activity and other properties of culture filtrates of staphylococci isolated from bovine mammary glands. R. RICHOU, MME. R. RICHOU, CL. GERBEAUX, J. JACQUET, and P. JULIENNE. Compt. rend. soc. biol., 146, 1493-1494. 1953. (Chem. Abstr., **47**: 7584g. 1953.) S. Patton

819. Microbiological assay of penicillin in animal feeds. R. C. KERSEY and F. V. LEGHORN, Chas. Pfizer & Co., Inc., Brooklyn. Appl. Microbiol., **1**: 150-152. 1953. (Chem. Abstr., **47**: 7135g. 1953.) S. Patton

820. The dehydrogenation of the fatty acids of milk by the action of various microorganisms. M. BENJAMBES and J. POISSON, Milk Research Sta., Paris. Ann. inst. natl. recherche agron., Sér. E, Ann. technol. agr., **1**, 1: 31-44. 1952. (Chem. Abstr., **47**: 8277d. 1953.)
S. Patton

DAIRY CHEMISTRY

821. Report on foreign fats in dairy products. The sterol acetate test. J. H. CANNON, Food and Drug Adm., Federal Security Ageney, St. Louis, Mo. J. Assoc. Off. Agr. Chemists, **36**, 2: 181-183. 1953.

A procedure is outlined for the sterol acetate test which has been successfully used to detect 5% of coconut oil in 95% of butterfat. A diagram is shown of a flask used in the procedure for handling the small amounts of precipitates. The author states that pure butter yields a residue having a m.p. no higher than 115.5° C. A mixture of hydrogenated coconut oil (5%) and butter (95%) gives a residue with a m.p. of 118.5° C. or higher. F. J. Babel

822. Titration of calcium and magnesium in milk and milk fractions with ethylenediamine tetraacetate. R. JENNESS, Dept. of Agr. Biochem., Univ. of Minn., St. Paul. Analyt. Chem., **25**, 6: 966-968. 1953.

The method of Schwarzenbach et al. for titrating calcium and magnesium with ethylenediamine tetraacetate and suitable indicators is used for water, limestone, soils and certain biological fluids. The method is simple, rapid and accurate, but it cannot be used with milk

because orthophosphate interferes with the endpoint. This paper describes a simple anion exchange technique for removing the phosphate from milk samples prepared for analysis by dry ashing, wet digestion, or acid precipitation of the casein. An aliquot of the solution thus prepared is passed through the anion exchange column to remove phosphate. Calcium and magnesium are determined on the effluent by titration. The titration is of comparable accuracy to the classical methods for determining calcium and magnesium, but the entire procedure including ion exchange is much less time consuming. B. H. Webb

823. Spectrochemical analysis of plant material using spark excitation. W. T. Mathis, Conn. Agr. Expt. Sta., New Haven, Analyt. Chem., **25**, 6: 943-947. 1953.

A spectrographic method of analysis for simultaneously determining all the major inorganic elements and the usual minor elements in alfalfa and other plant material from one exposure was developed. Acid extracts of the plant ash were used. Direct comparison of data obtained by this method with the results obtained by the more time-consuming chemical methods showed the new method to be equally reliable. B. H. Webb

824. Rapid modified procedure for determination of Kjeldahl nitrogen. C. H. Perrin, Canada Packers, Ltd., Toronto, Ontario. Analyt. Chem., **25**, 6: 968-971. 1953.

A rapid digestion method for Kjeldahl determinations is described. The digestion mixture of the proposed method is much like that of the Kjeldahl-Wilfarth-Gunning method, but with a few small modifications that increase the reaction rate several times. Improvement in digestion rate is attributed to use of a high concentration of mercury, intense heating and the use of silica granules. Data are given for use of the method on a variety of substances, including casein. B. H. Webb

825. Études Électrophorétiques du Lait. III. —Recherches sur le Lait de Vaches a la Fin de Lactation (Electrophoretic Studies of Milk III.—Research on bovine milk at the end of lactation). G. V. Heyndrickx and A. de Vleeschauwer. Bull Soc. Chim. Biol., **34**, 10: 1016-1025. 1952.

Electrophoretic patterns of centrifuged and dialized samples of milk at the end of lactation were obtained at pH 8 in sodium veronate buffer ($\mu = 0.1$). In general, a decrease was observed in the α- and β-casein fractions and in an additional casein fraction (C) with a mobility of $6.4 - 6.7 \times 10^{-5}$ cm^2 V^{-1} sec.$^{-1}$ An increase was noted in two of the lactalbumin fractions (D, E) and in the globulin fractions (H). A new fraction (B′) with a mobility between that of α-casein and fraction (C) also appeared in the milk at this time. This fraction has been tentatively identified as blood serum albumin by comparison of the electrophoretic patterns of the milk with those of serum albumin and mixtures of the two. The non-casein fractions (D, E, H) could be identical with certain blood serum fractions from a comparison of the electrophoretic patterns with those of blood serum and mixtures of the two.

R. McL. Whitney

826. Sammenhaengen mellem fedt- og proteinindholdet i maelk fra enkelte besaetninger. (The correlation between fat and protein content of milk from individual producers). K. P. Anderson, H. S. Rasmussen, and A. Herning, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull. 80: 53-71. 1953.

A standard formula for calculating the protein content of mixed milk on the basis of its fat content could not be applied to individual producers milk. The thought had occurred that daily deviations would be eliminated over a period of time and that such a formula then could be used when basing payment for milk on its protein content. Twelve producers were selected; samples of the individual milk as well as the mixed milk from all 12 producers were taken daily. The fat and protein content was determined weekly until each producer had delivered 7500 kg. of milk.

The calculations showed a mean deviation in the protein content of the mixed milk of $\pm$ 0.06%; for the individual producers milk the mean deviation was $\pm$ 0.11%, showing application of such a formula to be unsatisfactory.

T. Kristoffersen.

827. Analytische Bestimmung der Käsetrockenmasse durch Trochknung auf Folien. (Analytical Determination of total solid in cheese dried on foil.) (English Summary.) M. E. Schulz and G. Morwetz. Die Milchwissenschaft, **8**, 3: 93-95. 1953.

Five to 7 g. of cheese are weighed onto a sheet of aluminum foil of 0.015 mm. thickness and 150 × 190 mm. in dimensions. A similar second sheet is placed on top of the weighed cheese and by means of a suitable object having a smooth bottom surface, the cheese is pressed to 1 mm. thickness, covering an area of 100 mm. diameter. The two sheets of foil are separated and placed with the cheese side up in a drying oven at 105° C. After 1½-2 hrs. drying time, they are removed from the oven, placed together, allowed to cool, and reweighed.

I. Peters

828. Quantitative Vitamin D-Bestimmung in biologischen Test. (Biological quantitative determination of vitamin D.) (English summary.) H. Frahm. Die Milchwissenschaft, **8**, 3: 89-90. 1953.

Similar results were obtained when rats were fed vitamin D_2 or vitamin D_3. Chicks responded quicker to vitamin D_3 than to vitamin D_2, the sensitivity ratio being 1:12. Comparison feeding trials with rats and chicks permit, therefore, the differentiation between the two vitamins.

I. Peters

829. A presumptive crystallization test for milk fat adulteration. M. KEENEY, Dairy Dept., Univ. of Md., College Park. Ice Cream Trade J., **49**, 6: 30. 1953.

Milk fat crystallized from absolute ethanol exhibits distinct difference in some of the physical properties of the crystals. When a mixture of fat crystals and fat-alcohol solution is shaken, the crystals have a tendency to float on the alcohol solution in the case of milk fats, and a tendency to sink in the case of non-milk fats. Milk fat crystals entrain and hold air bubbles, causing the crystals to float, whereas non-milk fat crystals do not. A procedure based on these observations has been developed as a means of detecting the presence of non-milk fats in a mixture of fats. Five to 10% of non-milk fats may be detected by this method.

W. H. Martin

830. Microdetermination of copper in biological material. Improved dithizone-polarographic method. D. M. HUBBARD and E. C. SPETTEL, Kettering Laboratory, Dept. of Preventive Med. and Ind. Health, Coll. of Med., Univ. of Cincinnati, Ohio. Analyt. Chem., **25**, 8: 1245-1247. 1953.

An improved dithizone-polarographic method is described for the determination of copper in biological material. Samples are prepared for analysis by the standard wet-ashing procedure and copper is extracted by means of dithizone and finally determined polarographically.

B. H. Webb

831. Method of iodinating proteins and their derivatives. W. R. GRAHAM, JR., G. O. KOHLER, and R. D. HOOVER (assignors to American Dairies, Inc. and The Quaker Oats Co.). U. S. Patent 2,650,914. 2 claims. Sept. 1, 1953. Official Gaz. U. S. Pat. Office, 674, 1: 215. 1953.

Iodination of casein and other proteins is accomplished by the oxidation of I or I salts with alkali metal and alkaline earth metal hypochlorite in the presence of the protein.

R. Whitaker

832. Note on the determination of ash in non-fat dry milk solids. R. E. CANNY, Food and Drug Adm., Federal Security Agency, Minneapolis, Minn. J. Assoc. Off. Agr. Chemists, **36**, 2: 558-560. 1953.

Eighteen samples of neutralized and non-neutralized non-fat dry milk solids were analyzed in duplicate or triplicate by (a) ashing at 550° C. until free from carbon and (b) ashing for 1 hr. at 550° C., wetting with water, breaking up, drying on the steam bath and re-ashing for 1 hr. at 550° C. Statistical analysis of the data indicated no significant difference between the results of the two methods.

F. J. Babel

833. The thiobarbituric acid test for butterfat oxidation. D. A. BIGGS and L. R. BRYANT, Ont. Agr. College, Guelph. Canad. J. Technol., **31**, 6: 138-145. 1953.

The method for measuring oxidative deterioration in fat products proposed by Dunkley and Jennings has been investigated and modified. Time of reaction, pH of reaction mixture, presence of oxygen in the reaction chamber, and presence of an emulsifying agent in the reaction mixture are shown to influence the amount of color produced. The modified method can be applied to the determination of oxidation in powdered whole milk, Cheddar cheese, and butter. Interferences from colored materials in the sample can be eliminated by running blank determinations. Correction can be made for interference caused by lactose in milk powder by reference to a predetermined correction chart. Duplicate determinations on these products indicated good reproducibility. The results on milk powders showed a correlation between test values and organoleptic flavor scores and indicated that the test is capable of measuring degrees of oxidation below the level of organoleptic sensitivity.

O. R. Irvine

834. Pharmaceutical studies on enzymic hydrolyzates of proteins. III. Rate of liberation and hydrolysis of amino acids during the pancreatic digestion of casein. 1. KOUHEI FUKUDA, Univ. Kanazawa. J. Pharm. Soc. Japan, **73**, 327-329. 1953. (Chem. Abstr., **47**: 7564a. 1953.)

S. Patton

835. Rate of liberation and hydrolysis of amino acids during the pancreatic digestion of casein. 2. IBID. 329-331. (Chem. Abstr., **47**: 7564c. 1953.)

S. Patton

836. Microsomal particles of normal cow milk. R. K. MORTON, Univ. Cambridge, Engl. Nature, **171**, 734-735. 1953. (Chem. Abstr., **47**: 7580i. 1953.)

S. Patton

837. Nitrates in human milk. J. AMERICO PEZZANI. Rev. fac. cienc. quim., Univ. nacl. La Plata, **23**, 171-182. 1948. (Chem. Abstr., **47**: 7618h. 1953.)

S. Patton

838. Reductase of cow milk treated with ultrasonic waves. ALDO FERLAZZO, Univ. Messina, Italy and G. LOMBARDO. Riv. pediat. siciliana, **7**, 488. 1952. (Chem. Abstr., **47**: 7687d. 1953.)

S. Patton

839. Influence of cottonseed meal (in the ration) on the quality of milk and butter. I. Kokovkin. Molochnaya Prom., **14**, 5: 31-33. 1953. (Chem. Abstr., **47**, 7695g. 1953.) S. Patton

840. A hitherto unrecognized biochemical difference between human milk and cow milk. P. György, Univ. of Pa., Philadelphia. Pediatries, **11**: 98-108. 1953. (Chem. Abstr., **47**: 7053h. 1953.) S. Patton

841. The biochemical behavior of wheat-flour doughs supplemented with milk solids. A. L. Gordon, Univ. of Minn., Minneapolis. Univ. Microfilms, Ann Arbor, Mich., Publ. 4854, 161 pp. Dissertation Abstr., **13**: 169. 1953. (Chem. Abstr., **47**: 7129a. 1953.) S. Patton

842. Gravimetric and colorimetric determination of casein in cow milk. P. F. Fleury and R. Eberhard, Fac. pharm, Paris. Ann. pharm. franc., **11**: 183-187. 1953. (Chem. Abstr., **47**: 7129f. 1953.) S. Patton

843. The relation between fat and protein in milk. T. Lonka, Suomen Karjanjolostusyhdistys, Tikkuria, Finland. Maataloustieteellinen Aikakauskirja, **24**: 176-180. 1952. (Chem. Abstr., **47**: 7129h. 1953.) S. Patton

844. The lipolytic enzymes of raw skimmilk and separator slime. H. G. Nelson, Univ. of Minn., Minneapolis. Univ. Microfilms, Ann Arbor, Mich., Publ. 4869, 152 pp. Dissertation Abstr., **13**: 144-145. 1953. S. Patton

845. Phosphomonoesterases of cow milk. J. Jacquet and Odette Saingt, Lab. biol. et hyg., Caen, France. Compt. rend. soc. biol., **146**, 1515-1518. 1952. (Chem. Abstr., **47**: 8106e. 1953.) S. Patton

846. The structure of whey proteins. A. Lembke, W. Kaufmann, and Helga Schmidt, Forsch. Anstalt Milchwirtschaft, Kiel, Ger. Naturwissenschaften, **39**, 431. 1952. (Chem. Abstr., **47**: 8110a. 1953.) S. Patton

847. The heterogeneity of casein. Sh. G. Gonashvili. Inst. Animal Husbandry, Tiflis. Soobshcheniya Akad. Nauk. Gruzin. S.S.R., **10**, 519-526. 1949. (Chem. Abstr., **47**: 8114c. 1953.) S. Patton

848. The influence of various dyes on the flocculation of casein. L. Kesztyus and A. Surányi, Univ. Debrecen, Hung. Naunyn-Schmiedebergs Arch. exptl. Pathol. Pharmakol., **215**, 590-599. 1952. (Chem. Abstr., **47**: 8115d. 1953.) S. Patton

849. Influence of oxygen on irreversible denaturation of B-lactoglobulin. Gordon Johansen. Compt. rend. trav. lab. Carlsberg, Sér. chim., **28**, 335-341. 1953. (Chem. Abstr., **47**: 8133i. 1953.) S. Patton

850. The lipase activity of cow milk and various methods for its determination according to M. H. Peterson, M. J. Johnson, W. V. Price, B. C. Johnson, and I. A. Gould. D. Jacqmain, Lab. ind. aliment., Brussels, Belg. Rev. fermentations et inds. aliment., **5**, 18-21. 1950. (Chem. Abstr., **47**: 8164d. 1953.) S. Patton

851. New method for the determination of total solids and non-fatty solids in natural milk or rehydrated milk supplemented with vegetable fat. M. Ramos Cordova. Ciencia (mex.), **12**, 197-198. 1952. (Chem. Abstr., **47**: 8276f. 1953.) S. Patton

852. Seasonal variations in vitamin A and carotene content of cow milk. Stanislaw Berger, Z Dzialu Hig. Zywienia P.Z.H., Warsaw, Poland. Roczniki Panstwowego Zakladu Hig., **3**, 163-184. 1952. (English summary). (Chem. Abstr., **47**: 8276h. 1953.) S. Patton

853. The thiobarbituric acid test for butterfat oxidation. D. A. Biggs and L. R. Bryant, Ontario Agr. Coll., Guelph. Can. J. Technol., **31**, 138-145. 1953. (Chem. Abstr., **47**: 8278c. 1953.) S. Patton

DAIRY ENGINEERING

854. Frozen mix making device. P. Martin (assignor to Foster's Freeze National Corp.). U. S. Patent 2,645,911. 10 claims. July 21, 1953. Official Gaz. U. S. Pat. Office, 672, 3: 703. 1953.

A horizontal ice cream freezer having a spiral scraper blade attached to the shaft which follows in a spiral groove in the freezer cylinder wall. R. Whitaker

855. Method and apparatus for making a variegated frozen confection. F. L. Hackmann. U. S. Patent 2,646,757. 10 claims. July 28, 1953. Official Gaz. U. S. Pat. Office, 672, 4: 1016. 1953.

Ice cream, ice milk, etc., from a continuous freezer flows downward through an outer tube containing an inner concentric tube through which flows a material such as a fruit or chocolate syrup. The two streams make a right angle bend, where the inner tube terminates. A set of impeller blades, rotated by a shaft passing through a stuffing box, then breaks up the stream of syrup into individual portions which are distributed uniformly throughout the ice cream as it is discharged from the end of the tube. R. Whitaker

856. Apparatus for making refrigerated comestibles. G. E. READ. U. S. Patent 2,646,974. 9 claims. July 28, 1953. Official Gaz. U. S. Pat. Office, 672, 4: 1079. 1953.

Structural details are given for a vertical ice cream freezer with the outlet in the center below a collar which supports the end of the freezer shaft. Mix is introduced through an inlet on top. R. Whitaker

857. Float controlled valve. A. J. LIPPOLD (assignor to Cherry-Burrell Corp.). U. S. Patent 2,646,819. 3 claims. July 28, 1953. Official Gaz. U. S. Pat. Office, 672, 4: 1034. 1953.

A float valve of sanitary construction for controlling the inflow of a liquid into a tank. The supply line, entering from the top, terminates in a sleeve valve which is operated by a circular float surrounding the pipe and attached to the sliding sleeve. R. Whitaker

858. Self-measuring milk dispenser. H. T. COATES (assignor to Monitor Process Corp.). U. S. Patent 2,647,719. 5 claims. Aug. 4, 1953. Official Gaz. U. S. Pat. Office, 673, 1: 199. 1953.

An automatic, electrically operated dispensing unit for delivering a prescribed volume of milk into a glass from a can with an outlet in the side. R. Whitaker

859. Method of filling containers. R. KENNEDY, JR. (assignor to Abbotts Dairies, Inc.). U. S. Patent 2,648,476. 1 claim. Aug. 11, 1953. Official Gaz. U. S. Pat. Office, 673, 2: 482. 1953.

Details are given for the construction of a filler for continuously filling ice cream or other semiliquid products into containers. The container is lifted upward and filled first toward the forward wall and finally toward the rearward wall, the top surface being leveled and wiped clean by the wiping edge of the filling nozzle. R. Whitaker

860. Confection molding. C. R. NIKLASON. U. S. Patent 2,649,057. 3 claims. Aug. 18, 1953. Official Gaz. U. S. Pat. Office, 673, 3: 703. 1953.

Frozen novelties are produced by dipping refrigerated molds into containers holding mix until the mix has solidified. Defrosting is accomplished by raising the refrigerated mold with the novelty frozen to it, out of the container and detaching the novelty by raising the temp. of the mold. R. Whitaker

861. Removable seal for food processing equipment. W. H. LEUZE (assignor to Triumph Manufacturing Co.). U. S. Patent 2,649,317. 1 claim. Aug. 18, 1953. Official Gaz. U. S. Pat. Office, 673, 3: 775. 1953.

A sanitary type of stuffing box is described in detail.

862. Dispensing nozzle. F. T. MOSER (assignor to Limpert Brothers, Inc.). U. S. Patent 2,651,270. 1 claim. Sept. 8, 1953. Official Gaz. U. S. Pat. Office, 674, 2: 386. 1953.

Structural details are given for the construction of an ice cream filling machine nozzle, having as a novel feature a serrated peripheral outlet. R. Whitaker

863. Seal for centrifugal separators. G. J. STREZYNSKI (assignor to De Laval Separator Co.). U. S. Patent 2,651,455. 8 claims. Official Gaz. U. S. Pat. Office, 674, 2: 440. 1953.

Details are given for sealing the rotating bowl feed pipe of a centrifugal separator or clarifier to the non-moving supply pipe and bowl cover structure. R. Whitaker

864. Agitated milk pasteurizer. M. POPOW. U. S. Patent 2,650,980. 3 claims. Sept. 1, 1953. Official Gaz. U. S. Pat. Office, 674, 1: 230. 1953.

Electric heating elements are mounted in the space between two concentric cylindrical vessels and also within tubes which extend down into the milk in the inner vessel. Agitation is provided while the milk is heating, by reciprocating the cover of the vessel to which the heating tubes are attached. R. Whitaker

865. Extruder head. J. F. ECKLES and A. E. ECKLES, JR. U. S. Patent 2,650,551. 3 claims. Sept. 1, 1953. Official Gaz. U. S. Pat. Office, 674, 1: 114. 1953.

Ice cream or other semifluid product from 2 or more hoppers is proportionately blended and discharged from a series of outlets in the bottom of this extruder head. R. Whitaker

866. Apparatus for releasing frozen confections from molds. K. EFTIHIOS. U. S. Patent 2,649,764. 4 claims. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 960. 1953.

Molds from a brine tank are defrosted by hand in this equipment, which comprises a tank of warm defrosting liquid into which the mold is submerged to a controlled depth. Another tank surrounding the defroster collects the overflow. R. Whitaker

867. Receptacle filler and capper having receptacle carrier and capper indexing means. T. H. KUMMER and R. E. BODENDOERFER (assignors to Cherry-Burrell Corp.). U. S. Patent 2,650,012. 6 claims. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 1031. 1953.

Structural details are given for a hand operated milk bottle filler and capper.

R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

868. A comparative analysis of costs of farm collection of milk by can and tank in Western Washington, 1952. E. L. BAUM and D. E.

PAULS, Wash. Agr. Expt. Sta., Pullman. Tech. Bull. **10**: 1-37. 1953.

This bulletin discusses the important aspects of a farm tank system from a dealer's point of view. Some of the subjects mentioned are route length, number of shippers, route volume, distance between shippers, frequency of collection and labor requirements. R. W. Hunt

869. Bottle holder and carrier. B. H. LOCKE. U. S. Patents 2,647,786 and 2,647,787. 2 and 4 claims, respectively. Aug. 4, 1953. Official Gaz. U. S. Pat. Office, 673, 1: 218. 1953.

A carrier for glass milk bottles, consisting of a metal plate bent at an angle and provided with slots for sliding beneath the lip on the neck of the bottle. A handle is attached for carrying one or several bottles.

R. Whitaker

870. Method of remodeling milk cans. J. A. HOPWOOD (assignor to Monitor Process Corp.). U. S. Patent 2,647,302. 3 claims. Aug. 4, 1953. Official Gaz. U. S. Pat. Office, 673, 1: 79. 1953.

Details are given for making an outlet in the corner of milk cans, with the can bottom so shaped that the can slopes gently from the center to the outlet, thus providing complete drainage. R. Whitaker

871. King size cartons. B. GRANT. Ice Cream Field, **61**, 5: 14. 1953.

Data are presented to show that about 100,000,000 gal. of ice cream are annually distributed in ½-gal. cartons. Pints account for 48.4%, qts. for 12.2%, ½-gal. for 36.7% and gal. for 2.7% of the ice cream sold in linerless cartons. W. C. Cole

872. Ways of Lowering Milk Distribution Costs. D. E. HIRSCH, Farm Credit Administration, U. S. D. A. Milk Dealer, **42**, 10: 65-72. 1953.

Costs per unit per product may be reduced either by decreasing total cost or by increasing volume proportionately more than total cost. Labor is the most important single item in the cost of distributing milk. Increasing the volume of business usually is the most effective way of reducing labor cost per unit of product. Small plants tend to have higher costs per unit than larger plants. However, beyond a certain point, depending on current conditions, a plant may be too large and cost per unit will increase. There is a gradual drift toward a single quality standard for all milk for consumption in fluid form. Eventually, that standard also may apply to all or most of the milk used in the production of manufactured dairy products. This will encourage the interchangeability of milk, irrespective of final use, and may encourage the planned construction of plants designed to produce both kinds of dairy products. C. J. Babcock

873. Floor and Construction Materials. H. A. TONSAGER, Kraft Foods Co., Chicago, Ill. Butter, Cheese and Milk Prod. J., **44**, 8: 30-33. 1953.

A discussion of floor and construction materials for cheese manufacturing buildings is presented under the following headings: initial cost, local and/or governing building ordinances, space usage, appearance, maintenance and/or replacement costs, and sanitation requirements.

The most important problem, from an engineering standpoint, is to determine what the future holds for a particular plant so it is flexible enough to meet with the various operational demands. J. J. Jansen

874. Converting to In-Place Cleaning. J. R. GREENFIELD. The Diversey Corporation, Chicago, Ill. Milk Plant Monthly, **42**, 7: 22-24. 1953.

The operator who is contemplating installation of in-place cleaning should: (1) analyze present cleaning operations and determine the circuit or circuits to be used, (2) study processing layout and determine the circuit or circuits to be used, (3) consult an equipment supplier for information and advice on lines, special fittings, gaskets, solution tanks, pumps, thermometers, thermostats, etc. needed for the installation, (4) discuss plans for in-place cleaning with the inspecting authority and obtain approval, (5) be certain that the final plans conform to the recommendations set forth in "3-A Suggested Method for Installation and Cleaning of Cleaned-in-Place Sanitary Milk Pipelines in Milk and Milk Products Plants," and (6) obtain information on the cleaning and bactericidal treatment of the cleaned-in-place system from suppliers of detergents.

C. J. Babcock

FEEDS AND FEEDING

875. Growth depressing factors in rapeseed oilmeal. J. M. BELL and K. WILLIAMS, Univ. of Sask., Saskatoon. Canad. J. Agr. Sci., **33**, 3: 201-208. 1953.

Rapeseed oilmeal contains a water-soluble growth depressing factor. Growth rates in mice comparable to those obtained on linseed oilmeal supplemented rations were possible when the amount of rapeseed oilmeal was limited to 18%, or when 0.5% APF was added to the ration. No practical method of directly counteracting the action of the factor was found. Iodinated casein (0.1%) was ineffective in rations containing up to 27% rapeseed oilmeal, although it has been reported to be effective for fowl and rats.

These experiments suggest either species differences in response to the rapeseed goitrogen or the presence of more than one deleterious factor in rapeseed oilmeal. O. R. Irvine

876. Influence of iodized protein on the production and composition of cow milk. A. FRANCOIS, H. HEIM DE BALSAC, A. M. LEROY, and M. PAREZ, Natl. agron. inst., Paris. Ann. inst. natl. recherche agron., Sér. D, Ann. zootech., **1**, 1: 79-86. 1952. (Chem. Abstr., **47**: 8276i. 1953.) S. Patton

877. The use of the potato for feeding domestic animals. A. M. LEROY, Z. ZELTER, and R. FEVRIER, Natl. agron. inst., Paris. Ann. inst. natl. recherche agron., Sér. D, Ann. zootech., **1**, 1: 87-145. 1952. (Chem. Abstr., **47**: 8283g. 1953.) S. Patton

878. The study of a relationship of the dry-matter content, the nitrogen content, and the pH of a number of samples of pit silage. A. JOHN G. BARNETT, Univ. Aberdeen, Scot. J. Brit. Grassland Soc., **5**, 93-97. 1950. (Chem. Abstr., **47**: 8284b. 1953.) S. Patton

879. The conservation of grassland herbage in lined trench silos. A comparison of nutrient losses and the feeding value in molassed and ordinary silages. W. O. BROWN and I. H. HEANEY, Queen's Univ., Belfast, Ire. J. Brit. Grassland Soc., **6**, 91-98. 1951. (Chem. Abstr., **47**: 8284e. 1953.) S. Patton

880. Composition and quality of grass silages made in Northern Ireland. An analysis of eight years work (1940-1948). W. O. BROWN, Queen's Univ., Belfast, Ire. J. Brit. Grassland Soc., **5**, 225-235. 1950. (Chem. Abstr., **47**: 8284e. 1953.) S. Patton

881. The composition and nutritive value of three silages made from the same clover-grass crop. II. The silage. A. J. G. BARNETT, T. B. MILLER, and T. L. DODSWORTH, Univ. Aberdeen, Scot. J. Brit. Grassland Soc., **7**, 15-20. 1952. (Chem. Abstr., **47**: 8284g. 1953.) S. Patton

882. Nutritive value of cheatgrass and crested wheatgrass on spring ranges of Utah. C. WAYNE COOK and LORIN E. HARRIS, Utah Agr. Expt. Sta., Logan. J. Range Management, **5**, 331-337. 1952. (Chem. Abstr., **47**: 8284h. 1953.) S. Patton

883. Effects of grazing intensity upon the nutritive value of range forage. C. WAYNE COOK, L. A. STODDART, and LORIN E. HARRIS, Utah Agr. Expt. Sta., Logan. J. Range Management, **6**, 51-54. 1953. (Chem. Abstr., **47**: 8285a. 1953.) S. Patton

884. Nutritive value of browse on Montana winter ranges. DONALD A. JAMESON, Montana State Agr. Expt. Sta., Bozeman. J. Range Management, **5**, 306-310. 1952. (Chem. Abstr., **47**: 8285b. 1953.) S. Patton

885. Dairy-cattle feeding. Is urea the answer to protein concentrate shortages? P. MYLREA. Agr. Gaz. N. S. Wales, **64**, 9-10. 1953. (Chem. Abstr., **47**: 8285f. 1953.) S. Patton

886. Penicillin in milk replacements for dairy calves. C. B. KNODT and E. B. Ross, State College, Pa. Proc. Soc. Exptl. Biol. Med., **82**, 663-665. 1953. (Chem. Abstr., **47**: 7614e. 1953.) S. Patton

887. The effect of feeding poppy-extract "groats" and mixed cattle feed on the amount and the fat content of cow milk. J. LANGLET and G. COMBERG, Inst. Tierzucht Molkereiw., Halle, Ger. Tierzucht, **3**, 17-21. 1952. Chem. Zentr., 1950, II 717. (Chem. Abstr., **47**: 7615e. 1953.) S. Patton

888. Peanut cake in the nutrition of the milk cow. RAMON JAQUOT and PIERRE MERAT. Bull. mens. inform. ITERG, **7**, 165-180. 1953. (Chem. Abstr., **47**: 7617h. 1953.) S. Patton

889. Effect of heat blanching on alfalfa. C. R. THOMPSON, Western Regional Research Lab., Albany, Calif. Agr. Eng., **33**: 19-20. 1952. (Chem. Abstr., **47**: 7135d. 1953.) S. Patton

890. Engineers advance art of making grass silage. P. A. WHISLER and G. V. FRUSHOUR, Allis-Chalmers Mfg. Co., Milwaukee, Wis. Agr. Eng., **34**: 315-318. 1953. (Chem. Abstr., **47**: 7135f. 1953.) S. Patton

891. Drugs in animal feeds. H. E. MOSKEY, Food and Drug Admin., Washington, D. C. Food Drug Cosmetic Law J., **8**: 108-116. 1953. (Chem. Abstr., **47**: 7135h. 1953.) S. Patton

HERD MANAGEMENT

892. Economic aspects of farm tank handling of milk in Oregon. G. T. NELSON, Ore. State Coll., Corvallis. Ore. Expt. Sta. Cir. of Information **520**: 1-11. 1953.

This report is divided into six parts: (1) Planning for a tank system of handling milk; (2) Size and costs of farm tanks and refrigeration systems, methods of financing, and the farmer's attitude toward farm tanks; (3) Procedure used for picking up milk at the farm and savings in transporting milk from farm to the processing plant in tank trucks compared to can trucks; (4) Size of herd as a factor influencing costs of handling milk in cans and in tanks; (5) Comparative costs of receiving milk at the processing plant in cans and in tanks; (6) State of Oregon and City of Portland requirements for installation of farm tanks. R. W. Hunt

893. Changes in the dairy farming picture. W. E. McDaniel and C. A. Pond, Univ. of Minn., St. Paul. Agr. Expt. Sta. Bull. **416**: 1-19. 1953.

A review of the changes in operating a dairy farm in southeastern Minn. for the years 1920-1949. R. W. Hunt

894. The importance of machine milking rate in dairy cow management and breeding. F. H. Dodd and A. S. Foot, Natl. Inst. for Research in Dairying, Univ. of Reading, England. J. Dairy Research, **20**, 2: 138-145. 1953.

From the institutional herd at Reading 86 animals in about their 5th wk. of lactation were tested for rate of milking. The maximum rate attained in any min. during milking was analyzed for relationship to other characteristics of lactation such as daily milk yield and composition, standard lactation yield (305 d. or until dry if less than 305 d.), persistency, and lactation length.

For the 86 animals in the trial, the data for peak flow showed a mean and standard error of 4.04 ± 1.42 lbs. per min. with a coefficient of variation of 35.07%. This was the largest amount of variability shown for the variants recorded. Persistency and lactation yield showed the next largest degrees of variability.

It was found that the regression coefficients of peak flow on standard lactation yield was significant at the 1% level and that for each lb. per min. increase in flow rate there was 419 lbs. increase in total lactation yield. The partial regression coefficient of peak flow corrected for solids-not-fat and fat % to lactation milk yield also is highly significant. It is shown that peak flow is related to maximum daily yield (.9 lb. increase with each lb. per min. increase), lactation length (7.9 d. increase with each lb. per min. increase), and persistency (10.6 d. increase before production rate was ½ of maximum weekly rate for each lb. per min. increase). These relationships were significant at the 5% level.

The author's analysis by partial regressions indicates that the relationship noted between peak flow and standard yield is due to interactions and exists only because peak flow affects maximum daily yield, lactation length, and persistency.

The most important factor affecting standard lactation yield was firstly persistency then maximum daily yield and lastly lactation duration.

Data is presented indicating that milking rate is quite highly heritable and deserves much more consideration from genetisists than it has received in the past. J. D. Donker

895. The accuracy of measurements of the mammary glands by the method of palpation. D. Sheppard, Nat. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 2: 128-137. 1953.

The author has devised an objective technique to test the accuracy of the Swett udder palpation procedure. This was done by constructing artificial mammary glands of rubber tubing, blocked out in a frame of wood to hold a rectangular shape and then suspended in a sack of thick cloth. A series of 15 'glands' ranging from .85 to 5.45 cm. in length and in width from .85 to 3.70 cm. were palpated by the Swett method by 5 judges on 3 different occasions over a period of 6 mo., each judge giving replicate measurements of both widths and lengths at each trial for all 'glands.' These measurements then were compared to actual dimensions. For each subject there was a highly significant (.001) relationship between true size and measurements. The estimated sizes generally ran under true size and this varied in relationship to 'finger strength.' Those judges with stronger hands tended to show a greater deviation on the negative side than those with not so strong a grip. Estimated size was significantly different from true size and there was considerable variation. It was found that there was considerable difference among the subject's ability to 'measure glands.' Usually there was consistency between retrials, although certain judges showed a tendency to deviate further from the actual with replication and in later trials.

It is concluded that by use of this artificial gland, measurements taken by any one subject may be evaluated as to accuracy and repeatability, and that the results from this testing of technique would allow an estimate of size to be projected into true size so that the results of different judges may be compared with greater confidence. J. D. Donker

896. Stanchion for domestic animals. D. E. Ogden. U. S. Patent 2,648,308. 2 claims. Aug. 11, 1953. Official Gaz. U. S. Pat. Office, 673, 2: 431. 1953.

A stanchion for cows consisting of an inverted V-shaped yoke, suspended at the apex from an overhead frame, and closed on the bottom by a chain attached to the two arms. R. Whitaker

897. Teat protector for dairy cattle. R. E. Smith. U. S. Patent 2,649,073. 5 claims. Aug. 18, 1953. Official Gaz. U. S. Pat. Office, 673, 3: 707. 1953.

A device for protecting cows' teats consisting of a rubber ring surrounding the top of the teat which retains, by means of projections, an imperforate protecting cup made of rigid material. R. Whitaker

898. Claw for milking machines. H. W. Hein (assignor to International Harvester Co.). U. S. Patent 2,651,290. 7 claims. Sept. 8, 1953. Official Gaz. U. S. Pat. Office, 674, 2: 392. 1953.

A design for a claw or manifold of the pulsating vacuum type of milking machine.
R. Whitaker

899. Stock feeder. O. D. DUKE. U. S. Patent 2,651,291. 3 claims. Sept. 8, 1953. Official Gaz. U. S. Pat. Office, 674, 2: 392. 1953.

A feeder for dispensing mineral feed supplements to cattle, operated by the animal licking a roller having pockets which retain some of the dispensed supplement. R. Whitaker

900. Milk cooling and distributing means. E. MATHIESEN. U. S. Patent 2,650,427. 4 claims. Sept. 1, 1953. Official Gaz. U. S. Pat. Office, 674, 1: 79. 1953.

A cooler for farm use, consisting of a conical shaped jacketed vessel, the larger end on top, with a reservoir designed to distribute the milk over the inner surface of the cone after passing through a built-in filter. A refrigerant circulating through a spiral shaped coil in the jacket cools the milk. The cooled milk collects in a trough at the bottom small end of the cone and is directed through a tilting outlet pipe into one of two milk cans directly below the cooler.
R. Whitaker

901. Barn cleaner. F. W. VODOZ. U. S. Patent 2,646,158. 8 claims. July 21, 1953. Official Gaz. U. S. Patent Office, 672, 3: 774. 1953.

A motor driven nonportable device for cleaning the manure and litter from the gutter of dairy barns. An endless chain with impellers attached to it sweeps along the gutter and discharges the material collected into a dump truck. R. Whitaker

902. Milking machine pulsator. C. A. THOMAS (assignor to Babson Bros. Co.). U. S. Patent 2,646,058. 11 claims. July 21, 1953. Official Gaz. U. S. Pat. Office, 672, 3: 744. 1953.

Structural features are given for a hydraulic type pulsator for providing pulsations in the vacuum supply of a milking machine.
R. Whitaker

ICE CREAM

903. Shrinkage research — A final report. D. R. FRAZEUR and C. D. DAHLE, Pa. State Coll., State College, Pa. Ice Cream Field, **62**, 2: 50. 1953.

Caseinates but not casein are effective shrinkage deterents. Superheated sodium caseinate and powdered sodium caseinate were most effective in decreasing shrinkage. Caseinates allowed a higher overrun with batch freezers without increased danger of shrinkage.

Although albumin is a pro-shrinkage factor, high temperature pasteurization denatures the albumin to the extent that it may not contribute to shrinkage. Unaltered globulin is reported to be the anti-shrinkage factor of milk. Dialized Cheddar cheese whey yielded a globulin which was a powerful anti-shrinkage factor and albumin which was a powerful "pro-shrinkage" factor. It was pointed out that albumin is denatured by heating milk to high temperatures whereas globulin is only slightly denatured by the same treatment. Anti-shrinkage benefits resulted from the addition to the mix of condensed whey (preheated at 145° F., and superheated to 190° F. after condensing). The addition of colostrum to the mix decreased shrinkage. The anti-shrinkage properties of colostrum were increased as pasteurization temperatures were increased to 190° F.

Shrinkage was at a minimum when the mix was heated from 135 to 145° F. for 30 min. Heating from 145 to 160° F. for 30 min. gave the maximum shrinkage. Shrinkage decreased as the temperature of pasteurization increases from 160 to 190° F. for 30 min. The use of superheated concentrated milk products had a beneficial effect on shrinkage. Inactivation of enzymes either as a result of heat or by enzyme inhibitors was beneficial in preventing shrinkage.

Wheat sirups, sweetened condensed milk, emulsifiers, and eggs are listed as ingredients which increase shrinkage. Rancidity or the presence of free fatty acids also may increase the tendency towards shrinkage. Unlined and untreated paper containers increase shrinkage, whereas metal cans or aluminum foil liners decrease this tendency. W. C. Cole

904. Influence of water constituents (minerals) on the physical properties of whipping quality of ice cream mixes. F. L. FOUTS, W. A. KRIENKE, and L. E. MULL, Fla. Agr. Expt. Sta., Gainesville. Ann. Rept., p. 76. 1952.

Ice cream mixes were prepared of plastic cream and nonfat-dry-milk-solids as the only dairy ingredients, and sodium carboxymethylcellulose, sodium alginate, gelatin and nonstabilizer control as stabilizers. Hard water of approximately 300 p.p.m. total hardness and 155 p.p.m. sulfate iron, softened water (total hardness 5 p.p.m.), and distilled water was used. Irrespective of the stabilizer, the softened water resulted in mixes of lower titratable acidity. No appreciable viscosity differences were observed. Both distilled and soft water batches whipped considerably faster than the hard water mix. R. W. Hunt

905. New flavors for ice cream. W. A. KRIENKE and L. E. MULL, Fla. Agr. Expt. Sta., Gainesville. Ann. Rept. p. 80. 1952.

Injection and topping sauces have been prepared from fresh citrus fruit for ice cream. A decided interest by consumers was displayed for orange, lemon and lime ice cream. A blue colored sauce was prepared of coconut, which when it and cherry sauce was injected into the same basic ice cream, a resulting red, white and

blue product with a pleasant flavor and eye appeal was produced. R. W. Hunt

906. U. S. Public Health Service gives final okay to H.T.S.T. pasteurization of ice cream mix. D. T. FITZMAURICE and C. F. WEINREICH, Cherry-Burrell Corp. Ice Cream Trade J., **49**, 8: 28-29. 1953.

The U. S. Public Health Service approval is contained in an amendment to the definition of pasteurization in the Frozen Desserts Ordinance and Code. "The terms 'Pasteurization,' 'pasteurized' and similar terms shall be taken to refer to the process of heating every particle of mix to at least 155° F., and holding at such temperature continuously for at least 30 min., or to at least 175° F., and holding at such temperature continuously for at least 25 sec., in approved and properly operated equipment." W. H. Martin

907. The heavy metal content of gelling and stabilizing agents. J. C. BARTLET, E. LIST, M. PAGE, and R. A. CHAPMAN. Food and Drug Lab., Ottawa. Canad. J. Technol., **31**, 6: 146-153. 1953.

The arsenic, lead, zinc, and copper content of samples of agar, gelatin, Irish moss gelose, vegetable gums, and other stabilizing agents used in the food industry has been determined. Some samples of agar were found to contain appreciable quantities of zinc, which was not uniformly distributed, and therefore the sampling of shredded agar was studied. To obtain representative samples, a sampling tool was designed. Agar was found to contain as much as 450 p.p.m. of zinc and 16 p.p.m. of lead. Amounts up to 125 p.p.m. of zinc and 6 p.p.m. of lead were found in gelatin. Irish moss gelose (carrageen) contained less than 100 p.p.m. of zinc, up to 15 p.p.m. of lead, and 30 p.p.m. of copper. The amounts of lead, copper, zinc, and arsenic found in samples of sodium pectate, sodium alginate, liquid pectin, gum karaya, gum arabic (gum acacia), locust bean gum (carob gum), sodium carboxymethylcellulose, and methylcellulose are also given. With the exception of one sample of locust bean gum, no sample tested contained more than 2.0 p.p.m. of arsenic. One stabilizing mixture and one sample of sodium alginate contained excessive amounts of lead. O. R. Irvine

908. Report on frozen desserts. Preparation of sample, sucrose and acidity in ice cream. H. M. BOGGS, Food and Drug Adm., Federal Security Agency, Philadelphia, Pa. J. Assoc. Off. Agr. Chemists, **36**, 2: 190-194. 1953.

Three samples of strawberry ice cream containing 25% of 1 + 3 frozen fruits contained 18.75% fruit. When the ice cream was passed through a 30-mesh screen and weighed, the fruit recovered amounted to 8.1, 13.0 and 10.0%.

Results of 5 out of 7 collaborators were in excellent agreement on the sucrose content of a sample of ice cream (14.12 to 14.86%). The average values obtained by the other 2 collaborators were 13.26 and 16.22%.

No data were presented on acidity of ice cream. F. J. Babel

909. Continuous ice cream freezer and method of making ice cream. W. L. MURPHY (assignor to De-Raef Corp.). U. S. Patent 2,651,186. 10 claims. Sept. 8, 1953. Official Gaz. U. S. Pat. Office, 674, 2: 362. 1953.

A system of freezing ice cream which freezes precooled mix containing incorporated gas. The mix is precooled by mixing it with the gas which has been expanded and chilled in the heat exchanger-type of freezer. R. Whitaker

910. Fruit-containing frozen confections and process for producing the same. W. F. TALBURT and D. G. GUADAGNI (assignors to the United States of America, as represented by the Secretary of Agriculture). U. S. Patent 2,651,575. 14 claims. Sept. 8, 1953. Official Gaz. U. S. Pat. Office, 674, 2: 475. 1953.

Soft textured pieces of fruit, impregnated with a full flavored fruit juice concentrate, are used with a flavored sugar solution to form a base for freezing into a frozen dessert similar to an ice. R. Whitaker

911. Process for making candies, confections, icings, and the like and the resulting product. E. O. WHITTIER (dedicated to the People of the United States). U. S. Patent 2,651,574. 9 claims. Sept. 8, 1953. Official Gaz. U. S. Pat. Office, 674, 2: 475. 1953.

A method is described for developing body in candy, confections, icing, etc., without heat. Crystallized sugar and dry flavoring materials are mixed with a dried milk product containing lactose in the amorphous form. Water or other aqueous liquid is added with stirring to form a paste which develops body as the lactose crystallizes to form the hydrate. R. Whitaker

912. Apparatus for making composite ice cream confections. E. L. ELWELL (assignor to Eskimo Pie Corp.). U. S. Patent 2,649,744. 3 claims. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 955. 1953.

This piece of equipment consists of two supply tanks and gear pumps. Freezer fresh ice cream is pumped from one tank through the outside of two concentric tubes to continuously surround a core of fruit, chocolate or other filling pumped from the other tank through the inner tube. R. Whitaker

913. Top billing to ice cream. A. BLUMENTHAL. Ice Cream Trade J., **49**, 8: 30-32. 1953.

Today 49% of the more than 19,000 indoor

theatres sell ice cream or roughly 9,500 houses (about three times as many as in 1949) and 92% of the 4,150 drive-ins or approximately 3,900 of them sell the product.

Preference of ice cream items by the patrons were listed as follows: 58% preferred ice cream on a stick; 42% cups and sandwiches; 34% cones; 16% chocolate covered ice cream; and 15% snow cones.

Preference percentagewise of the top nine items sold in a drive-in were as follows: popcorn 40%; soft drinks 38%; franks 19.5%; candy 19%; coffee 12%; ice cream 11%; and hamburgers, nuts and gum range between 4 and 6%. W. H. Martin

914. Nation-wide report on mellorine. Robert C. Hibben, Ex. Sec'y., IAICM. Ice Cream Trade J., **49**, 6: 22. 1953.

The problem with respect to Mellorine has been, for the last several years, "What policy will best promote honesty and fair dealing?" The IAICM Committee on Definitions and Standards has prepared a Model Mellorine Law for states wishing to introduce Mellorine, a Model Ice Cream Law for states wishing to strengthen their ice cream standard, and a Model Filled Dairy Products Act for states not wishing to introduce Mellorine. The situation as of May 22, 1953, the virtual close of the legislative season, was: 8 states permitting the sale of Mellorine; 8 states with Model Filled Dairy Products Acts on the books; 3 states with new Model Ice Cream Laws; 2 states with Mellorine legislation pending; and in one state legislation dealing with Mellorine was killed. W. H. Martin

915. First analysis of the volume in Mellorine. Ice Cream Trade J., **49**, 8: 22-23. 1953.

Eleven million gal. of Mellorine, the vegetable and/or animal fat type frozen dessert, was produced in 1952 in Illinois, Missouri, Oklahoma and Texas. Current production in 1953 is at a higher rate, because 1952 production in some states did not start until after the first of the year. There were 317 plants in the 4 states producing mellorine in 1952.
W. H. Martin

916. Half-gallon potential. A. C. Ragnow, Fairmont Foods Co., Omaha, Neb. Ice Cream Field, **61**, 5: 26. 1953.

There has been a trend towards factory-filled package ice cream. Fairmont Foods Co. in 1952 packaged more than half of its ice cream for take home trade. During the past two years there has been increased emphasis on the ½ gal. package. W. C. Cole

MILK AND CREAM

917. Preserving raw milk with various antibiotic preparations. V. W. Greene and J. M. Bell, Univ. of Sask., Saskatoon. Sci. Agr., **32**, 12: 619-625. 1952.

The preservative action of a number of antibiotics when added to fresh raw milk and skimmilk reconstituted from powder was studied with a view to using these materials for self-feeding baby pigs. Acidity determinations were used chiefly to assess preservative action and the milk samples were incubated up to 24 hours at 37° C. The following materials were used: aurofac, terramycin, streptomycin, sulfathaladine, sulfaguanidine, penicillin, aureomycin, APF, and ustilagic acid. Aureomycin and terramycin were found to inhibit acid production and bacterial growth for 20 hrs. at rates of about 10 p.p.m. The sulfas were effective at 1000 p.p.m. Penicillin and aurofac, while rather effective separately, exerted a marked effect in combination in conc's. of 10 p.p.m. and 1.0% respectively. O. R. Irvine

918. The effect of residual penicillin in milk on the dye reduction tests for quality. C. K. Johns and J. G. Desmarais, Science Service, Canada Dept. of Agr., Ottawa Canad. J. Agr. Sci., **33**, 1: 91-97. 1953.

Confirming earlier findings, results by the methylene blue test were frequently found to be affected to an appreciable degree by small conc's. (0.05 and 0.5 units/ml.) of penicillin added to raw milks. Because of its shorter incubation period, results by the resazurin "triple reading" test were affected to a much lesser extent. The report that resazurin results were affected more when milks containing penicillin were refrigerated overnight was investigated but the effect was found to be too slight to affect the results. O. R. Irvine

919. Variations in the fat content of milk throughout the milking process. W. G. Whittlestone, Ruakura Animal Research Sta., Dept. of Agr., Hamilton, New Zealand. J. Dairy Research, **20**, 2: 146-153. 1953.

The author, from observations made on the changes of first drawn and last drawn milks from cows and sows and the creaming properties of these respective milks, has proposed a theory to account for the phenomena that the last drawn milk of the cow is so much richer in fat than the first drawn milk. In the sow there is no difference between the first and the last drawn milk. Further, sows milk does not cream when allowed to set. However, if sow's cream is centrifugally separated and added to cow's skimmilk the cream will rise quickly. While on the other hand cow's cream added to sow's skimmilk will rise only after a prolonged time.

It is postulated by the author that agglutinins present in cow milk serum cause a clustering of the fat globules, and that the clustering effect impedes the flow of milk fat as distinguished from the less viscous portion. The agglutinins are not present in the sow and therefore the fat content of the milk is uniform throughout the flow period. J. D. Donker

920. Report on fat in dairy products. Variations by different technicians in estimating upper meniscus on the fat column of the Babcock test for milk. E. O. HERREID, Ill. Agr. Expt. Sta., Urbana. J. Assoc. Off. Agr. Chemists, **36**, 2: 183-185. 1953.

The results of 16 technicians who tested 1426 samples of milk indicate significant variations in estimating the dimensions of the upper meniscus on the fat column of the Babcock test. The average per cent of fat in the meniscus ranged from 0.05 to 0.20%, depending on the technician. F. J. Babel

921. Report on fat in homogenized milk. A modified Babcock method. C. E. HYNDS, State Food Laboratory, Dept. of Agr. and Markets, Albany, N. Y. J. Assoc. Off. Agr. Chemists, **36**, 2: 185-186. 1953.

A proposed modified Babcock test for homogenized milk is outlined. The proposed method was compared with the standard Babcock method and another modified method (acid added in either 3 or 4 portions and shaken 30 to 45 sec. after each addition) in 4 laboratories. Data showing the percentage variance from the Roese-Gottlieb test are given for all methods.
F. J. Babel

922. Preparation of sample of pressurized cream. C. G. CUNNINGHAM, Food and Drug Adm., Federal Security Agency, Boston, Mass. J. Assoc. Off. Agr. Chemists, **36**, 1: 128-131. 1953.

A method is presented for the preparation for analysis of samples of whipped toppings in pressurized containers. The method involves freezing the contents of the container, releasing the gas while contents are frozen, transferring the frozen contents to a Waring blendor, and mixing thoroughly. Replicate determinations of fat and total solids indicate that a uniform sample is obtained. A representative sample was not obtained by dispensing a well shaken product as directed on the can.
F. J. Babel

923. Usefulness of the lactometer at temperature below 50° F. W. A. KRIENKE, Fla. Agr. Expt. Sta., Gainesville. Ann. Rept. p. 79. 1952.

Studies revealed that only slight errors are involved when lactometer readings are taken as low as 38° F. and converted to 60° F. by use of the accepted formula. R. W. Hunt

924. Applications of several antibiotics to the food industry. II. Applications of several antibiotics to cow milk. 2. WATARU HASHIDA, Osaka Univ. J. Fermentation Technol. (Japan) **31**: 15-18. 1953. (Chem. Abstr., **47**: 7130d. 1953.) S. Patton

925. Applications of several antibiotics to the food industry. III. Application to the preservation of cow milk. 3. WATARU HARSHIDA and TOSHIAKI ASAI, Osaka Univ. J. Fermentation Technol., (Japan) **31**, 112-117. 1953. (Chem. Abstr., **47**: 8276g. 1953.)
S. Patton

926. Estimation of the freshness of milk by means of resazurin and methylene blue. MIROSLAWA WEBER, Z Oddzialu Badania Zyunosei i Przedmiotów Uzytku Filii P.Z.H., Kraków, Poland. Roczniki Panstwowego Zakladu Hig., **3**, 2: 185-196. 1952. (French Summary) (Chem. Abstr., **47**: 8277e. 1953.)
S. Patton

927. Canning whole fresh milk. JOHN C. MOERI (to Farm Fresh Milk Corp.). U. S. 2,642,363, June 16, 1953. (Chem. Abstr., **47**: 8286i. 1953.) S. Patton.

928. Relation between penicillin content of cow milk and lactobacilli after injection of penicillin. TOYOKI MIYABE and YOSHIKAZU HIGASHI. Tech. Bull Kagawa Agt. Coll., **4**, 136-140. 1952-53. (Chem. Abstr., **47**: 7686d. 1953.) S. Patton

929. Flocculated milk. M. LUST. Lattante, **23**, 385-391. 1952. (Chem. Abstr., **47**: 7687i. 1953.) S. Patton

930. Multi-Vitamin-Mineral fortified milk. J. KLEIN, General Mills, Inc., New York. Sou. Dairy Prod. J., **54**, 2: 107. 1953.

Multi-vitamin-mineral fortified milk is a comparatively new product. It is milk in which vitamins A, B_1, B_2, C, D; niacin, calcium, phosphorus, iron and iodine are balanced to meet the minimum daily nutritional requirements of consumers. It is being marketed as fortified homogenized milk at a premium of 1½ or 2¢ per qt. in Wisconsin, Indiana, Illinois, Kansas, Oklahoma, Arizona, Minnesota, Iowa and California. F. W. Bennett

931. Selection of Nonfat Dry Milk Solids Used in the Preparation of Buttermilk. W. M. ROBERTS and L. M. BLANTON, N. C. State Coll. Milk Plant Monthly, **42**, 7: 29-30. 1953.

In the preparation of buttermilk from nonfat dry milk solids use nonfat dry milk solids of extra grade quality; adjust the concentration of solids to give the desired viscosity in the finished buttermilk—10% usually gives a medium bodied product; mix with water at 70°-100° F. in a sanitary manner; pasteurize at 180° F. for 30 minutes and cool to 70° F.; add 1% of good active starter and ripen to an acidity of .85 to .90%; with controlled agitation, cool quickly to 40° F.; allow to stand for 1 to 2 hrs. at 40° F. for the air to escape and then bottle. Butter flakes or pasteurized cream

and salt (.5 to 1 lb. per 100 gal.) can be added to the buttermilk during the cooling period.

C. J. Babcock

MILK SECRETION

932. Untersuchungen über die Laktation von Nichtmilchschafen mit vergleichenden Betrachtungen zur Laktation von Kühen. (Comparison of the lactation of non-milking breeds of ewes with the lactation of cows). (English summary.) A. Ulrich. Die Milchwissenschaft, **8**, 3: 96-98, **4**: 130-132. 1953.

The concentration of milkfat, protein, and lactose in milk of Leine ewes and cows followed a similar pattern during the course of lactation. Fluctuations in the concentration of individual milk components in individual animals followed a similar pattern between ewes and cows. This was shown by examining first and last-drawn portions of milk from the same milking, the milk of adjacent quarters and by varying the frequency of milkings.

In ewe's milk the negative correlation of fat: protein: lactose was more pronounced than in cow's milk. In samples from individual ewes, however, the variation of composition was of the same order as in cow's milk. I. Peters

NUTRITIVE VALUE OF DAIRY PRODUCTS

933. Nutritive value of butterfat compared with that of vegetable fat. I. Biological experiments. A. Experimental results. C. Nieman, E. H. Groot, and B. C. P. Jansen, Netherlands Inst. Natl. Nutrition, Amsterdam. Proc. Koninkl. Ned. Akad. Wetenschap, 55C: 587-597. 1952. (Chem. Abstr., **47**: 7048b. 1953.) S. Patton

933. B. Discussion of experimental results. Ibid. 598-604. (Chem. Abstr., **47**: 7048c. 1953. S. Patton

933. II. Statistical analysis. Ibid. 605-615. (Chem. Abstr., **47**: 7048d. 1953.) S. Patton

934. Vitamin B_{12} (cobalamin) content of the milk of lowland and hill sheep. D. H. Shrimpton and J. Duckworth, Rowett Research Inst., Aberdeen, Scot. J. Sci. Food Agr., **4**, 301-304. 1953. (Chem. Abstr., **47**: 8277b. 1953.) S. Patton

935. Clinical determination of vitamin D. in ultraviolet irradiated milk. Otto Hovels, Univ. Frankfurt am Main, Ger. Z. Kinderheilk., **72**, 646-657. 1953. (Chem. Abstr., **47**: 7578a. 1953.) S. Patton

936. Nutrition research and the dairy industry. Zoe E. Anderson, Nat'l. Dairy Council. Sou. Dairy Prod. J., **54**, 2: 65-67. 1953.

Retaining a market for food products will depend upon the development of products on a nutritional basis and upon the public appreciation of the nutritional values of the foods. Since 1940 the National Dairy Council has administered a research program for the dairy industry, including research projects in 20 colleges with the purpose of supplying a continuous flow of information to meet these two essential needs.

The Council functions as a liaison agent by evaluating research proposals, developing research ideas, consulting with investigators, making recommendations, handling details essential to cooperation and interpreting research reports for industry and the public. The research projects to be supported through the Council are selected upon their importance and where the research is to be done. The available physical facilities and the training and experience of personnel are considered in the location of projects.

The nutrition research program includes nutrient content and digestibility of dairy foods, utilization and human requirements for nutrients in dairy foods, comparison and interrelationship of nutritional values, consumer acceptance and nutrition education.

F. W. Bennett

PHYSIOLOGY AND ENDOCRINOLOGY

937. Reviews of the progress of Dairy Science. Section A. Physiology of dairy cattle. I. Reproduction and lactation. J. A. B. Smith, The Hannah Dairy Research Inst., Kirkhill, Ayr, Scotland. J. Dairy Research, **20**, 2: 224-253. 1953.

The author has reviewed the subject matter from the year 1949 to the present. This review covers a total of 324 articles pertinent to the field covered. The most important papers have been well reviewed and abstracted and much useful information is found including the author's interpretation and philosophical viewpoints. J. D. Donker

938. Mineral balances at parturition as related to occurrence of parturient paresis in dairy cows. G. M. Ward, T. H. Blosser, and M. F. Adams, Wash. Agr. Expt. Sta., Pullman. Inst. of Agr. Sci. Tech. Bull. **5**: 1-24. 1953.

The work described in this bulletin was divided into two sections: (1) mineral balances and blood levels of cows at time of parturition and (2) pretreatment and past-treatment levels of some blood constituents of cows developing parturient paresis. When calcium intake and excretion was averaged by groups and a balance calculated, it was found that the cows which developed milk fever had more severe negative calcium balances for a longer period prior to calving than did other groups of cows. Sodium balance was not significantly different and

chlorine balances were available only on four cows. The average potassium balances for the cows developing milk fever and for the first calf heifers were greatly different. The primiporous group was in positive balance throughout the study, while the animals developing milk fever were in a negative balance at all times. No explanation is offered for this difference. The second phase of the study involved ten cows which developed clinical symptoms of parturient paresis. Calcium, sodium, potassium, and chloride analyses were made on blood samples only. The mean of all pretreatment levels of sodium was slightly lower, potassium slightly higher, and chloride levels considerably higher. The same trend was found for both normally calving and milk fever cows in the first portion of this study. Therefore, the causes for these differences remain obscure.
R. W. Hunt

939. Acclimatization to low oxygen tension. J. C. Strickney and E. J. Van Liere. Dept. Physiol., School of Med., W. Va. Univ., Morgantown. Physiol. Rev., **33**, 1: 13-34. 1953.

Studies relating to the adjustment of animals (including man) to atmospheres of low oxygen tension such as high altitudes, have been reviewed. Changes taking place in the lungs, blood, and organs during acclimatization were discussed. 156 references cited.
E. G. Moody

940. Volume and distribution of blood and their significance in regulating the circulation. Torgny Sjöstrand, Lab. Clin. Physiol., Karolinska Sjukhuset, Stockholm, Sweden. Physiol. Rev., **33**, 2: 202-228. 1953.

The author discussed the physiological and pathological variations in the blood volume as well as the effect of blood volume and blood distribution upon circulation. 124 references were cited.
E. G. Moody

941. Biochemistry and physiology of epidermis. C. Carruthers and V. Suntzeff. Washington Univ. Med. School, St. Louis, Mo. Physiol. Rev., **33**, 2: 229-243. 1953.

The review was focused upon the epidermis alone and did not include the associated sebaceous glands, hair follicles, sweat glands, or other accessory structures. The author described the epidermis, its development and replacement, mineral composition, enzyme activity, vitamine and related compounds, amino acid and protein relationships. 136 references cited.
E. G. Moody

942. Indirect determination of water retention in cattle as a means of estimating energy exchange. K. L. Blaxter and J. A. F. Rook, Hannah Dairy Research Inst., Kirkhill, Ayr, Scot. Nature, **171**, 609-610. 1953. (Chem. Abstr., **47**: 7578g. 1953.) S. Patton

943. Biosynthesis of milk fat in the rabbit from acetate and glucose. The mode of conversion of carbohydrate into fat. G. Popjak, G. D. Hunter, and T. H. French, Natl. Inst. Med. Research, London. Biochem. J., **54**, 238-247. 1953. (Chem. Abstr., **47**: 7623i. 1953.) S. Patton

944. Electrophoretic study of semen. T. Erdos. Acta Physiol. Acad. Sci. Hung., **3**: 525-536. 1952. (Chem. Abstr., **47**: 6999e. 1953.) S. Patton

945. Changes in blood composition of cows during lactation as related to varying nutritional levels. G. A. Bondarenko. Zhur. Obshchei Biol., **13**: 464-678. 1952. (Chem. Abstr., **47**: 7052b. 1953.) S. Patton

946. The distribution of concentrations of bicarbonate (including carbon dioxide) and chloride in the omasum of cows. J. Ekman and I. Sperber. Kgl. Lantbrukshögskolans Ann., **19**: 227-231. 1952. (in English). (Chem. Abstr., **47**: 7058h. 1953.) S. Patton

947. The flow properties of cervical secretions in the cow as related to certain physiological conditions. F. A. Glover and G. W. Scott Blair, Univ. Reading, Engl. J. Endocrinol., **9**: 160-169. 1953. (Chem. Abstr., **47**: 7062h. 1953.) S. Patton

948. The heat of combustion of the tissues of cattle in relation to their chemical composition. K. L. Blaxter and J. A. E. Rook, Hannah Dairy Research Inst., Kirkhill, Ayr, Scot. Brit. J. Nutrition, **7**: 83-89. 1953. (Chem. Abstr., **47**: 7064b. 1953.) S. Patton

949. The physiological chemistry of calcium and phosphorus metabolism of young cattle. F. Almasy, A. Krupski, and H. Ulrich, Univ. Zurich, Switz. Bull. schweiz. Akad med. Wiss., **8**: 450-475. 1952. (Chem. Abstr., **47**: 7067g. 1953.) S. Patton

950. Chemical studies of mammalian sperm. R. Duncan Dallam and Lloyd E. Thomas, Univ. of Missouri, Columbia. Biochem. et Biophys. Acta, **11**, 79-89. 1953. (Chem. Abstr., **47**: 8159e. 1953.) S. Patton

951. Change in the concentration of reducing sugar in bovine fetal fluids during gestation. Thomas S. Matney, M. C. Chang, and John B. Loefer, Trinity Univ., San Antonio, Texas. Growth, **15**, 213-224. 1951. (Chem. Abstr., **47**: 8220b. 1953.) S. Patton

952. Additional information on the pharmacological action of oleum terebinthinae on the uterus of cattle. Grzegorz Staskiewicz, Univ. Marie Curie Sklodowskiej, Lublin, Poland.

Ann. Univ. Marie Curie-Sklodowska Lublin, Poland, Section DD, **6**, 407-416. 1951. (English Summary) (Chem. Abstr., **47**: 8259i. 1953.) S. Patton

SANITATION AND CLEANSING

953. Cleaned-in-Place. H. E. Calbert, Univ. of Wis. Milk Plant Monthly, **42**, 7: 15-20. 1953.

Cleaned-in-place lines in dairy plants should be installed according to the recommendations of the 3-A committee, and the 1953 milk ordinance and code of the U. S. Public Health Service. Many of these recommendations apply for the installation of these lines on dairy farms. The dairy farm pipe line probably can be installed at a lower cost if it is to be cleaned by a vacuum method, than if it is to be cleaned by a pressure system. Provisions should be made so that at least sections of the farm pipe line would be dismantled for periodic inspection. The cleaning of pipe lines both in dairy plants and on dairy farms should receive utmost attention at the time of installation. The manufacturer of the pipe line and the manufacturers of cleaning and sanitizing agents should be consulted for instructions on cleaning. Diagrams are presented showing the installation of permanent pipe lines in stanchion type barns and in milking parlors. C. J. Babcock

954. Report on extraneous materials in dairy products. D. B. Scott, Food and Drug Adm., Federal Security Agency, Washington, D. C. J. Assoc. Off. Agr. Chemists, **36**, 2: 301-307. 1953.

A discussion of the histology of plant material is presented to serve as a guide for the identification of plant fragments and to aid in determining whether the fragment is from dung or compost, or is undigested plant tissue. The procedure for the Graichen-Harrow staining method is given. The staining procedure is used to make the characteristics of the fragments more easily observed. F. J. Babel

955. Report on sediment tests in milk and cream. C. R. Joiner, Food and Drug Adm., Federal Security Agency, St. Louis, Mo. J. Assoc. Off. Agr. Chemists, **36**, 2: 310-315. 1953.

Standard disks prepared from different sediment mixtures varied slightly in appearance but the differences were insignificant. A real difficulty was that of getting the sediment evenly distributed over the disk and a suitable filtering apparatus was necessary for this purpose. Wetting the filter disk before filtering the sediment suspension was essential. The relatively thin "cream-test" disks were usually more satisfactory for the fine standard pads than "milktest" disks because more sediment was embedded in the thicker pad. A description is given of a proper type of sediment disk filtering apparatus for preparing standard disks. F. J. Babel

956. Fly control with a new bait application method. R. K. Thompson, A. A. Whipp, D. L. Davis, and E. G. Matte, Research and Development Dept., Calif. Spray-Chemical Corp. J. Econ. Entomol., **46**, 3: 404-409. 1953.

Insecticidal bait tests against the housefly in dairy barns were conducted in 1951 and 1952. Either lindane, TEPP (tetraethyl pyrophosphate), or a combination of the two was mixed with water and some attractant for application by means of a garden sprinkling can. Four to 8 oz. of either syrup, molasses, dried milk, whey, or calf starter were added to 10 qts. of the mixture; corn syrup was best.

The bait was sprinkled in "wet strips," immediately after mixing, on clean floor surfaces of the barn where flies usually congregated. Applications were best made after the morning barn-cleanup. During cool periods, better results were obtained by sprinkling later in the day.

Bait containing 0.15% lindane plus 0.06% TEPP, applied at 2 to 3 d. intervals was effective. It also controlled hornflies on those cattle which remained standing on the treated floor about one hour at each milking. Neither toxicant alone was nearly as effective.

The greatest hazard with this bait material lies in handling the original concentrate (10.7% lindane plus 4.3% TEPP). At the use dilution there appears to be no hazard to domestic animals which may be in the barn. Lindane or TEPP was not found in milk from cows in treated barns. E. H. Fisher

957. Torsalo and tick control with toxaphene in Central America. E. W. Laake. J. Econ. Entomol., **46**, 3: 454-458. 1953.

The most common and important external parasites of cattle in Central America are the torsalo, *Dermatobia hominis* L., and the fever tick, *Boophilus annulatus* var. *microplus*. The former is generally found only in Central and South America; it has become the worst insect enemy of cattle where it is well established. Recently it was found in a dog brought to Texas from Central America. Stable, horn, and house flies are carriers of torsalo eggs.

Sprays or dipping vat charges containing 0.5% toxaphene, made from either wettable powder or emulsifiable solution, have been effective against both pests. Dipping in 0.37% toxaphene also was effective.

Investigations by U. S. D. A. entomologists indicate the amount of toxaphene excreted in milk of cows thus treated, if any, would not make the milk unfit for human consumption.

E. H. Fisher

958. Enzymatic dehydrochlorination of DDT by resistant flies. J. STERNBURG, E. B. VINSON, and C. W. KEARNS, Univ. of Ill., Urbana. J. Econ. Entomol., **46**, 3: 513-515. 1953.

Enzyme extracts from DDT—susceptible flies did not dehydrochlorinate DDT to DDE, but those from a DDT-resistant strain did. It was suggested that DDE is a metabolic intermediate.

E. H. Fisher

959. Method of and apparatus for washing milking machines. A. E. ANDERSON. U. S. Patent 2,650,179. 5 claims. Aug. 25, 1953. Official Gaz. U. S. Pat. Office, 673, 4: 1078. 1953.

The teat cup assemblies of a milking machine are easily cleaned in this device. One assembly is placed in each of 2 containers, which are connected together by a tube passing through the covers of each container. The assembly tubes are attached, on the inside of the containers, to the ends of the connecting tube. Cleaning fluid is placed in one container to a depth to cover the assembly. Air pressure is applied to that container which forces the cleaning liquid through the assemblies into the other container. The process is then reversed and repeated many times. The alternating flow of the liquid and agitation supplied by the bubbles of air at the end of each cycle facilitate cleaning the milking machine parts.

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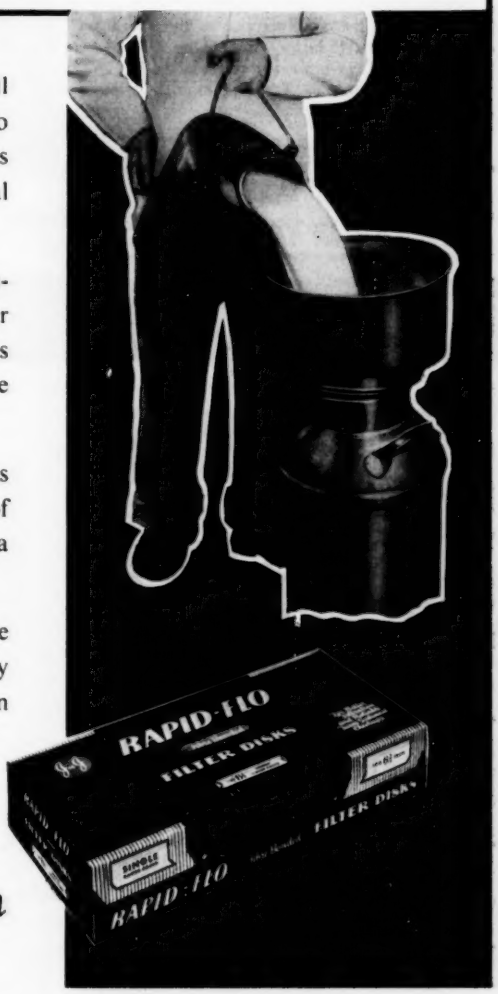

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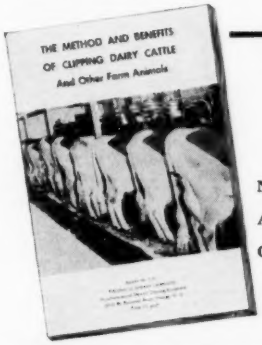

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